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 DATE 2-13-04

IN THE UNITED STATES DISTRICT COURT
 FOR THE
 DISTRICT OF MASSACHUSETTS

CIVIL ACTION NO: _____

SERONO, INC., and
 INDUSTRIA FARMACEUTICA SERONO SpA,

Plaintiffs,

v.

FERRING PHARMACEUTICALS, INC.

Defendant.

JURY TRIAL DEMANDED

COMPLAINT

MAGISTRATE JUDGE Alexander

04 CV 10305 MLW

Plaintiffs, Serono, Inc. and Industria Farmaceutica Serono S.p.A. (hereinafter collectively "Serono"), present this Complaint of patent infringement against defendant, Ferring Pharmaceuticals, Inc. (hereinafter "Ferring"). Serono is the innovator and patent holder for a number of important inventions to help infertile couples who are trying to have a baby. The patent rights at issue in this lawsuit cover pharmaceutical hormone compositions and therapeutic regimens that are used for ovulation induction and in vitro fertilization. Defendant Ferring is actively infringing Serono's patent rights in the United States, as set forth below in further detail.

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JURISDICTION AND PARTIES

1. This is a civil action for patent infringement arising under Title 35 of the United States Code.
2. Jurisdiction and venue are proper in this judicial district pursuant to 28 U.S.C. §§ 1331, 1338(a), 1391 and 1400(b).
3. Plaintiff Serono, Inc. is a Delaware corporation having a principal place of business at One Technology Place, Rockland, MA 02370.
4. Plaintiff Industria Farmaceutica Serono S.p.A. is an Italian corporation having a principal place of business in Rome, Italy.
5. Defendant Ferring Pharmaceuticals, Inc. is a Delaware corporation with a principal place of business at 400 Rella Boulevard, Suffern, NY 10901.

FACTUAL BACKGROUND

6. On February 16, 1988, the United States Patent and Trademark Office duly and lawfully issued to Serono, Inc. (then known as Serono Laboratories, Inc.) United States Letters Patent No. 4,725,579 ("the '579 patent") entitled *Method of In Vitro Fertilization by a Unique Combination of Gonadotropins*. The '579 patent generally covers methods for improving the recruitment of oocytes for *in vitro* fertilization through a regimen of administering follicle stimulating hormone, or "FSH," and luteinizing hormone, or "LH," in specified ratios. A copy of the '579 patent is attached hereto as Exhibit A.
7. On June 16, 1998, the United States Patent and Trademark Office duly and lawfully issued to Industria Farmaceutica Serono S.p.A. (then known as Istituto di Ricerca Cesare Serono S.p.A.) United States Letters Patent No. 5,767,067 ("the '067 patent") entitled

Follicle Stimulating Hormone and Pharmaceutical Compositions Containing Same. The '067 patent generally covers highly purified FSH preparations and pharmaceutical compositions containing such highly purified FSH preparations. A copy of the '067 patent is attached hereto as Exhibit B.

8. The '579 patent was developed out of research sponsored by Serono at the Eastern Virginia Medical School. The co-inventors, Drs. Howard and Georgeanna Jones, were two of the pioneers in the treatment of infertility, particularly in developing *in vitro* fertilization techniques that are used in clinics and hospitals throughout the world today. As discovered by the Joneses, administration to women of a ratio of 1.5:1 to 4:1 FSH to LH (as opposed to the prior art formulations that were generally 1:1 ratios of FSH to LH) substantially improved the percentage of cycles with a transfer of concepti, increased the number of total eggs and preovulatory eggs, and improved pregnancy rates overall.

9. The '067 patent, which resulted from research conducted in-house at Serono, describes and claims a highly purified FSH preparation which was a dramatic improvement over the FSH preparations known in the prior art. At the time of the invention, FSH was typically prepared by purifying the hormone from women's urine, and it was a very difficult compound to purify. Pertinent prior art compounds typically contained FSH together with LH and significant amounts of other urinary proteins. These prior art FSH compounds were known in the art as "pure" FSH because, although small amounts of LH were present, the amount was insufficient to produce LH bioactivity. The improved, highly purified FSH of the '067 patent contained no detectable LH based on immunoassay standards described in the '067 patent and was also substantially free of other urinary proteins. The administration of the highly purified FSH of the '067 patent was viewed as highly desirable because it would allow self-administration by

subcutaneous injection as well as careful regulation of FSH intake without unwanted LH. The compound claimed in the '067 patent, therefore, was a substantial improvement in the art of fertility treatment.

10. Serono sells pharmaceutical products containing FSH and/or mixtures of FSH and LH, and these products are used in the methods claimed in the '579 patent. Ferring sells products containing FSH and/or mixtures of FSH and LH, called respectively Bravelle® and Repronex®, and these products are used illegally in the methods claimed in the '579 patent. The '579 patent expires on February 21, 2005. Because of the short period of time left on the term of this patent, the remaining period of exclusivity is very important to Serono's infertility product lines.

11. The '067 patent, which covers highly purified FSH, expires on March 30, 2015. In its website, Ferring describes Bravelle® as "a highly purified human-derived FSH." Moreover, Ferring recently produced non-confidential documents in *Serono, Inc. v. Ferring*, Civil Action No. 02-11832 MLW (D. Mass), in which Ferring's President, Wayne Anderson, represented to the public that Bravelle® has "recombinant hormone-like purity." FPI 7746 (Exhibit C). An FSH product having "recombinant hormone-like purity" does not contain LH.

12. Similarly, Ferring recently produced non-confidential documents in which a Ferring scientist, Dr. Dennis C. Marshall, represented to the public that Bravelle® contains no LH. *See* Marshall et al., "Mixed-protocol, same-syringe combinations of gonadotropins: compatibility of new, highly purified, human-derived FSH (Bravelle®) and hMG (Repronex®)" *Today's Therapeutic Trends* 2001, 19, 213-224, at 220 (FPI 2721-2731 at 2727) (Exhibit D).

13. Ferring's Bravelle® formulation of highly purified FSH falls squarely within the scope of the claims of the '067 patent, which call *inter alia* for "follicle stimulating hormone

preparation free from traces of luteinizing hormone detectable at 1.5 mIU/ml, based on the 2nd IRP-HMG reference standard for luteinizing hormone. . . .”

14. The FSH in Bravelle® clearly has the correct FSH amino acid sequence specified in the claims of the '067 patent, as evidenced by Ferring's Bravelle® product insert:

Bravelle™ is a product containing a highly purified preparation of human follicle stimulating hormone (hFSH) extracted from the urine of postmenopausal women. Human FSH consists of two non-covalently linked glycoproteins designated as the α and β subunits. The α subunit has 92 amino acids of which two are modified by attachment of carbohydrates. The β subunit has 111 amino acids of which two are modified by attachment of carbohydrates.

Ferring Product Insert (Exhibit E).

15. In the mixed protocol article discussed above, Dr. Marshall and, by extension, Ferring, teaches the co-administration of Bravelle® with Repronex®, containing a ratio of 150:75 FSH:LH, (actual ratio = 169.21:83.13 FSH:LH) or roughly a 2:1 ratio. This practice falls squarely within the ratios for co-administration of FSH and LH set forth in claim 1 of the '579 patent (“ratio of about 1.5:1 to 4:1 total I.U. FSH to LH”). See Marshall et al., Today's Therapeutic Trends 2001, 19, 213-224 at 220 (FPI 2721-2731 at 2727) (Exhibit D). This article is one of several annotated references highlighted in the literature that Ferring uses to teach its customers how to use its products.

16. Indeed, Ferring's press releases and the programs used in Ferring presentations confirm that Ferring has induced its customers to practice the co-administration of these compounds in ratios which would infringe the '579 patent. See Ferring Press Release: “FDA Approves Bravelle” (FPI 007877); Ferring Press Release: “Ferring Presents data on New Infertility Treatments at the Pacific Coast Reproductive Society Meeting” (FPI 007918); and American Society of Reproductive Medicine (ASRM) Annual Meeting prospectus (Exhibit F)

(confirming that Ferring has promoted the co-administration of these compounds “to a targeted audience of empowered buyers in the field of reproductive medicine.”)

FIRST COUNT

(INFRINGEMENT OF THE '579 PATENT)

17. Plaintiffs repeat and re-allege the allegations in paragraphs 1 through 16 of this Complaint as though set forth here in full.

18. Ferring and its agents have used and have provided instructions for customers to use Bravell® and Repronex® in this Commonwealth and other States in the United States in manners that would infringe one or more claims of the '579 patent. On information and belief, customers have used Bravelle® and Repronex® pursuant to Ferring's instructions in this Commonwealth and other States in the United States in a manner that infringes at least one or more claims of the '579 patent.

19. Such conduct infringes one or more claims of the '579 patent under 35 U.S.C. § 271(a), and Ferring is liable for inducing infringement of the '579 patent under 35 U.S.C. § 271(b).

20. Ferring's infringing activity has been and will continue to be done in willful disregard of Plaintiffs' patent rights.

SECOND COUNT

(INFRINGEMENT OF THE '067 PATENT)

21. Plaintiffs repeat and re-allege the allegations in paragraphs 1 through 16 of this Complaint as though set forth here in full.

22. Ferring and its agents have sold and offered for sale Bravelle® in this Commonwealth and other States in the United States, which infringes one or more claims of the

'067 patent. Ferring's customers have used Bravelle® in this Commonwealth and other States in the United States.

23. Such conduct infringes one or more claims of the '067 patent under 35 U.S.C. § 271(a). Such conduct by Ferring's customers infringes one or more claims of the '067 patent under 35 U.S.C. § 271(a), and Ferring is liable for inducing said infringement of the '067 patent under 35 U.S.C. § 271(b).

24. Ferring's infringing activity has been and will continue to be done in willful disregard of Plaintiffs' patent rights.

RELIEF REQUESTED

WHEREFORE, Plaintiffs pray for judgment and relief including:

- (A) A judgment that United States Patents Nos. 4,725,579 and 5,767,067 are valid and enforceable;
- (B) A judgment that Ferring has been and is infringing one or more claims of United States Patents Nos. 4,725,579 and 5,767,067 in violation of 35 U.S.C. § 271 by its acts of importation, use, offering to sell, and/or sale of its highly purified FSH product and its FSH/LH mixture product in the manner explained above prior to expiration of these patents;
- (C) A preliminary and permanent injunction pursuant to 35 U.S.C. § 283, enjoining Ferring and its officers, agents, servants, employees, privies, and others acting for, on behalf of, or in concert with any of them from infringing of any claims of the patents in suit;
- (D) An accounting and award of Plaintiffs' damages caused by Ferring's infringement;

- (E) An award trebling the damages pursuant to 35 U.S.C. § 284 as a result of factors in this case, including Ferring's willful infringement of the patents in suit;
- (F) An award declaring this case exceptional pursuant to 35 U.S.C. § 285 and granting Plaintiffs their attorneys fees in pursuing this case;
- (G) An assessment of costs and prejudgment interest to Plaintiffs; and
- (H) Such other and further equitable relief as this Court may deem just and proper.

JURY DEMAND

Pursuant to Fed. R. Civ. P. 38, Plaintiffs demand a trial by jury on all issues that are properly triable to a jury in this action.

Dated: February 13, 2004


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BOS1353815.1



US005767067A

United States Patent [19]

Arpaia et al.

[11] **Patent Number:** 5,767,067[45] **Date of Patent:** Jun. 16, 1998[54] **FOLLICLE STIMULATING HORMONE AND PHARMACEUTICAL COMPOSITIONS CONTAINING SAME**[75] **Inventors:** Guiseppe Arpaia; Serenella Serani; Antonino Sirna; Stefano Villa, all of Rome, Italy[73] **Assignee:** Istituto di Ricerca Cesare Serono S.p.A., Rome, Italy[21] **Appl. No.:** 413,936[22] **Filed:** Mar. 30, 1995**Related U.S. Application Data**[60] **Continuation of Ser. No. 767,297, Sep. 27, 1991, abandoned, which is a division of Ser. No. 337,766, Feb. 7, 1989, Pat. No. 5,128,453.**[30] **Foreign Application Priority Data**

Jun. 26, 1987 [IT] Italy 48110/87

[51] **Int. Cl.⁶** C07K 14/59; A61K 38/24[52] **U.S. Cl.** 514/8; 530/398; 514/12; 930/110[58] **Field of Search** 530/398; 514/8; 514/12; 930/110[56] **References Cited****U.S. PATENT DOCUMENTS**

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Donini et al (II), "Purification and Partial Chemical-physical Characterization of FSH from Menopausal Urine". (E&S Livingstone, 1970) pp. 39-56.

Donini, "Recent Data on the Chemistry of Human Gonadotropins". *The Endocrine Function of the Human Testis (Academic Press, 1973) pp. 195-221.***Primary Examiner**—Vasu S. Jagannathan**Assistant Examiner**—Christine Saoud**Attorney, Agent, or Firm**—Browdy and Neimark

[57]

ABSTRACT

Purification of human FSH from post-menopausal urine gonadotropin using immunochromatography and reverse phase HPLC steps yields a biologically active hormone which is free from detectable traces of LH and other urinary proteins.

10 Claims, 3 Drawing Sheets

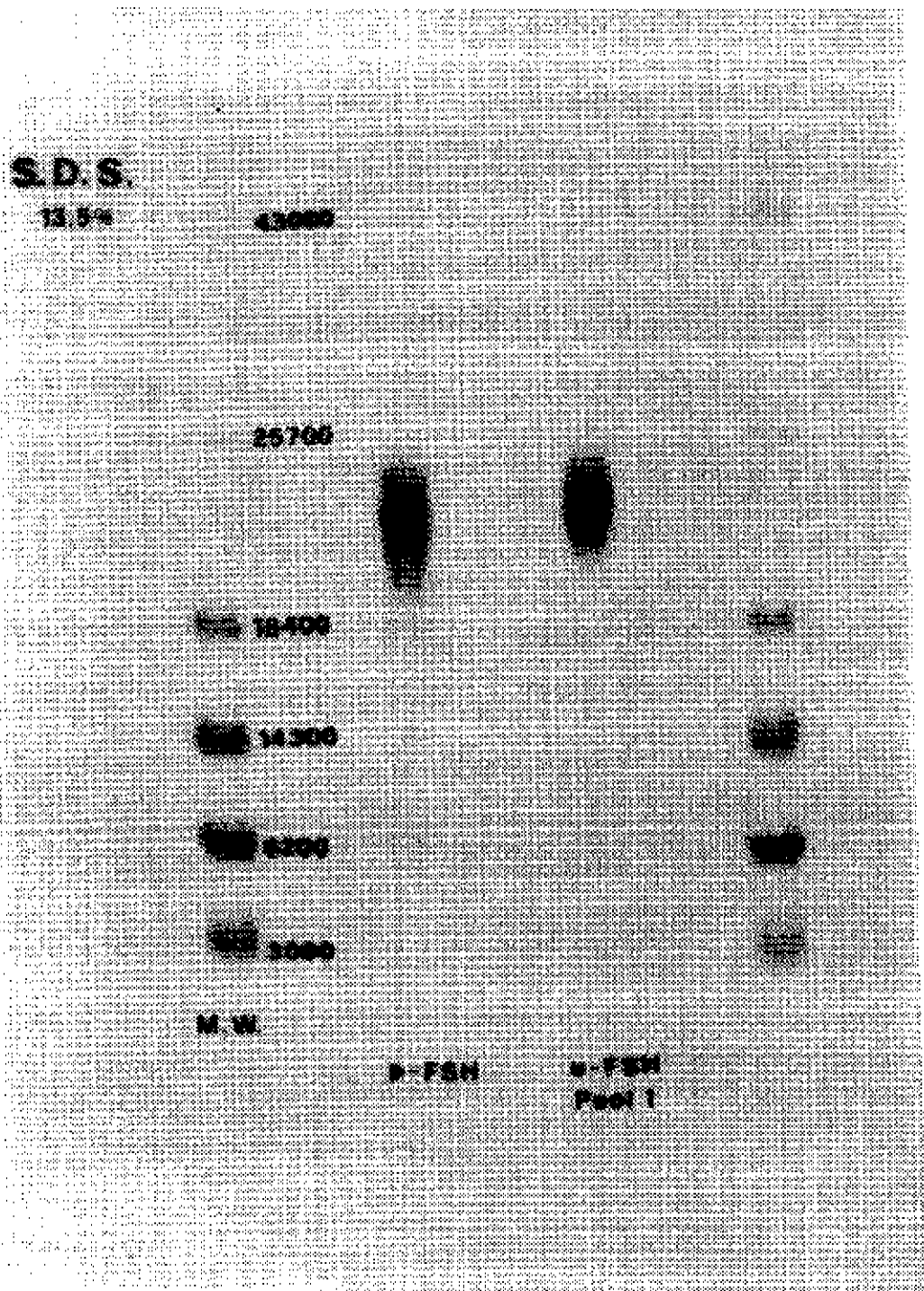
U.S. Patent

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FIG. 1



U.S. Patent

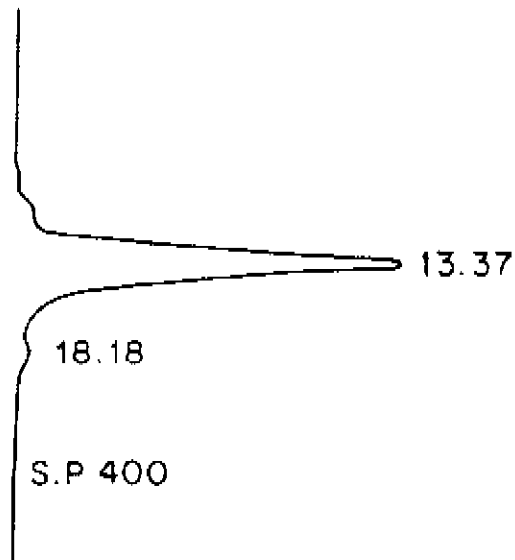
Jun. 16, 1998

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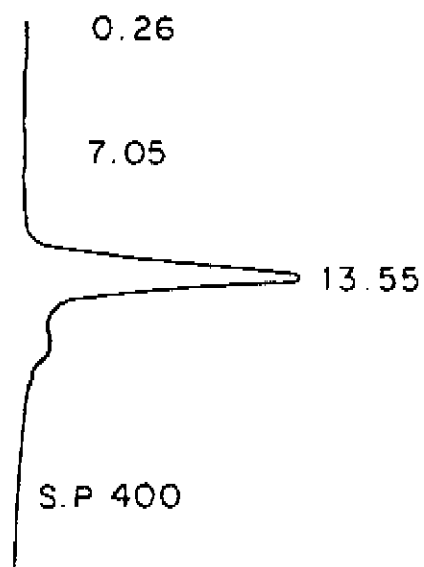
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FIG. 2

uFSH



pFSH



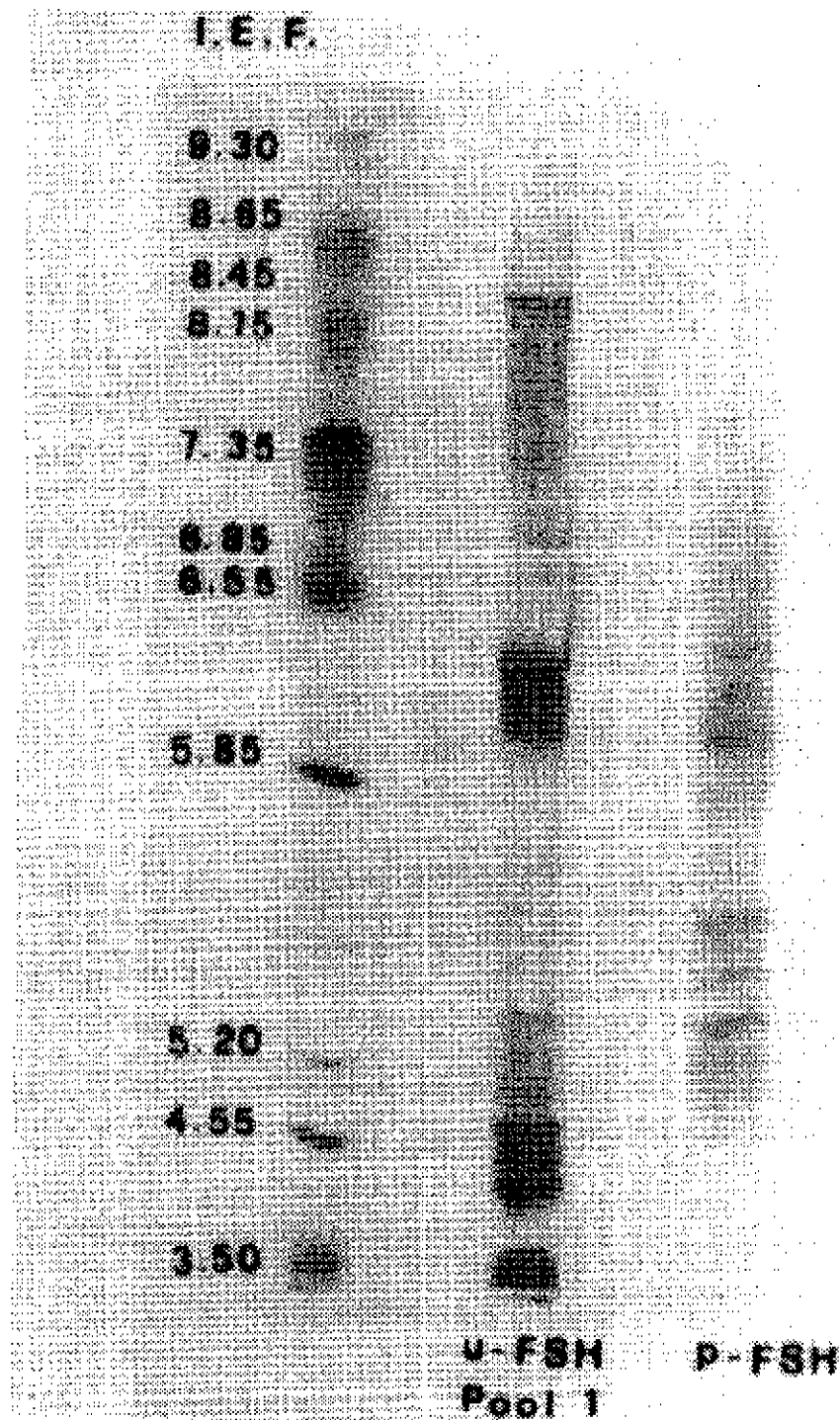
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FIG. 3



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FOLLICLE STIMULATING HORMONE AND PHARMACEUTICAL COMPOSITIONS CONTAINING SAME

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of application Ser. No. 07/767,297, filed Sep. 27, 1991, now abandoned, which was a division of application Ser. No. 07/337,766, filed Feb. 7, 1989, now U.S. Pat. No. 5,128,453, which application was a 371 of PCT/IT88/00048, filed on Jun. 24, 1988.

FIELD OF THE INVENTION

This invention relates to substantially pure biologically active follicle stimulating hormone, to pharmaceutical compositions containing it and to a method for its purification.

BACKGROUND OF THE INVENTION

Follicle stimulating hormone (FSH) is known to be useful in the treatment of infertility. Preparations containing this hormone have been employed to assist in effecting pregnancy using both in-vivo and in-vitro techniques. Human FSH has been isolated from human pituitary glands and from post-menopausal urine. More recently, it has been produced using recombinant DNA techniques.

The first commercially available product comprising human FSH contained HMG (e.g., Pergonal® Serono), i.e., human menopausal gonadotropin extracted from post-menopausal urine which is a mixture of FSH, Luteinizing Hormone (LH) and other urinary proteins. Meanwhile, several attempts were made to obtain pure FSH preparations, both for scientific and therapeutic purposes. The product Metrodin® (Serono), currently available in commerce, is a preparation of urinary FSH containing other urinary proteins, but minimum quantities of LH and is used for the treatment of infertility. Use of this product is particularly advantageous when administration of exogenous LH together with FSH is undesirable, e.g., in the polycystic ovary syndrome (PCOS).

So far, administration of FSH for therapeutic purposes has been carried out, successfully, exclusively by intramuscular injection. Since intramuscular injections are generally performed by the physician or by the medical professional staff, the patient is expected to visit a surgery or a hospital regularly in order to receive the treatment. This creates a considerable discomfort. Moreover, the time taken up by this type of application often leads to unsatisfactory compliance by the patient as the treatment normally extends over several weeks or months.

Administration by subcutaneous injection would render possible the self-administration by the patient and consequently improve patient's cooperation and compliance.

The subcutaneous administration of Human Menopausal Gonadotropin (HMG) has already been described (Nakamura Y. et al., *Fertility and Sterility*, 46(1):46-54, 1986) in connection with the treatment of female infertility by pulsatile administration of HMG via the subcutaneous peristaltic pump. The subcutaneous administration may suffer the drawback of the appearance of local allergies due to the presence of impurities in the product used and, consequently, result in the suspension of the treatment.

P. Roos ("Human Follicle Stimulating Hormone", *Acta Endocrinologica Supplementum* 131, 1968) described and characterized highly purified preparations of pituitary and urinary FSH obtained from frozen pituitaries and from

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post-menopausal urinary concentrate, respectively. Biological potencies as high as about 14,000 I.U. of FSH activity per mg for pituitary FSH and 780 I.U. of FSH activity per mg for urinary FSH were obtained. The content of LH contamination in the most active pituitary and urinary preparations was estimated to correspond to approximately 0.1 per cent by weight. The purification procedures involved one or more of such techniques as chromatography on DEAE-Cellulose, gel-filtration on Sephadex G-100, hydroxylapatite chromatography, polyacrylamide gel electrophoresis, and the like.

One of the best purified urinary FSH preparations was described by Donini et al. ("Purification and partial Chemical-physical characterization of FSH from Menopausal Urine", *Gonadotrophins and Ovarian Development* (Proceedings of two workshop meetings), E and S Livingstone, Edinburgh and London, 1970) and had a biological potency of 1255.6 I.U. FSH per mg with an LH contamination as low as 3.2 I.U. LH per mg. In this case, the starting material was a Human Menopausal Gonadotropin (HMG) preparation (Pergonal®) which, as stated above, is a mixture of FSH and LH hormones and other urinary proteins. This result was achieved by batchwise purification of the starting HMG on DEAE-Cellulose followed by chromatography on a DEAE-Cellulose column, gel filtration on Sephadex G-100 and a final step of preparative polyacrylamide gel electrophoresis.

Even higher biological potencies were achieved by H. Van Heli et al. ("Purification and some properties of human urinary FSH and LH", *Gonadotrophins* (Proceedings of Int.1 Symposium on Gonadotrophins, 1971), Wiley-Interscience, New York) by adding immunochromatography and gel-filtration steps to a conventional chromatographic purification procedure. Immunochromatography was performed using anti-HCG antibodies coupled to Sepharose for the specific purpose of removing the LH activity from partially purified FSH fractions. The best purified FSH fraction contained 4720 I.U. FSH per mg and the LH contamination was as low as 15 I.U. LH per mg as assayed by RIA.

The immunochromatographic approach had already been described by Donini et al. ("Purification and Separation of FSH and LH from HMGU", *Acta Endocrinologica* 52, pages 186-198, 1966) who obtained as early as 1966 an FSH preparation which had a potency of 329.7 I.U. FSH per mg and biologically undetectable amounts of LH contamination. In another experiment, a fraction assaying 148.3 I.U. FSH per mg and 2.4 I.U. LH per Mg was obtained. Since no RIA was performed on the 329.7 I.U. FSH per mg preparation, it can be assumed by analogy with the results of H. Van Heli (c.f. supra) that traces of LH would have been found if assayed by RIA.

The physiological relevance of even minimal amounts of LH contamination was shown by Donini et al. in a paper ("A new approach to the Biological Determination of the Luteinizing Hormone", *Acta Endocrinologica*, 58, pages 463-472, 1968) where a new bio-assay for LH determination was proposed which consisted in injecting intact immature mice with a constant dose of a biologically pure FSH preparation plus increasing amounts of LH and then measuring the increase in the uterine weight as a response proportional to the LH activity. By this method, as little as 0.068 I.U. of LH were shown to be capable of increasing the uterine weight when injected together with 4.44 I.U. of FSH.

It thus clearly follows that an absolutely pure FSH preparation is desirable when FSH activity in the complete absence of LH activity is requested in therapy.

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As mentioned above, the currently marketed product Metrodin® (Serono) is a purified FSH preparation which is obtained by a method substantially identical to that described by Donini et al. in an already mentioned paper (*Acta Endocrinologica* 52, pages 186-198, 1966). The quality control specifications, in agreement with the declared biological purity of the said preparation, provide for not more than 0.7 I.U. LH per 75 I.U. of FSH, i.e., approximately the sensitivity limit of the biological assay. In certain therapeutic applications, however, the absolute absence of LH is desirable. Furthermore, in Metrodin®, human FSH is accompanied by substantial amounts of other urinary proteins, i.e., it is not a chemically pure FSH preparation.

U.K. patent application 2,173,803 A provides still another approach to the purification of pituitary glycoprotein hormones, among them FSH. This approach consists of first forming a complex of the hormone with immobilized monoclonal antibodies and then eluting the hormone with an acidic aqueous buffer having a pH from 3 to 4. As far as FSH is concerned, the obtained highly purified hormone is still contaminated with 0.1% by weight of LH and 0.5% by weight of TSH.

Scott C. Chappel et al. describe in a review article (*Endocrine Reviews* 4(2), 179-211, 1983) the microheterogeneity of FSH and the physiological significance of the carbohydrate moieties accompanying the FSH molecule. The hypothesis can be formulated that some modifications occur during the metabolic pathway up to the final urinary secretion which may account for the chemico-physical differentiation demonstrated in the experiments carried out by Applicant between the highly purified urinary FSH preparation (hpuFSH) according to this invention, and the highly purified pituitary FSH preparation used for comparison purposes.

Co- or post-translational modifications appear to be the basis for microheterogeneous diversity of FSH, a glycoprotein consisting of two dissimilar, glycosylated, non covalently linked polypeptide chains known as alpha and beta-subunit. The beta-subunit endows the molecule with its biological specificity.

Incidentally, the same article attempts to explain the substantial difference in the values of biological activity per mg between highly purified pituitary and urinary FSH preparations. A reasonable hypothesis can again be based on the relevance of the carbohydrate moieties and, more specifically, the role of the terminal sialic acid residues.

It should be noted that, while both pituitary FSH and urinary FSH are termed "FSH", no conclusive evidence has yet been found which verifies whether the molecules isolated from the two sources are the same or even chemically equivalent.

DESCRIPTION OF THE INVENTION

It has now been found that human urinary FSH can be isolated from a concentrate of post-menopausal urine to such a degree of purity that the resultant FSH is completely free from any detectable traces of LH and other urinary proteins. Furthermore and contrary to any expectation, the amino acid analysis of the urinary FSH according to the present invention has revealed a novel FSH beta-subunit of 111 amino acids instead of 118 or 108 as reported in the state of the art (see e.g. Scott et al. supra) for the known FSH beta-subunit. More specifically, this invention provides a novel FSH beta-subunit, the amino acid sequence of which is as follows:

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      10
Asn Ser Cys Glu Leu Thr Asn Ile Thr Ile Ala Ile Glu
      20
Lys Glu Glu Cys Arg Phe Cys Ile Ser Ile Asn Thr Thr
      30
Trp Cys Ala Gly Tyr Cys Tyr Thr Arg Asp Leu Val Tyr
      40
Lys Asp Pro Ala Arg Pro Lys Ile Glu Lys Thr Cys Thr
      50
Phe Lys Glu Leu Val Tyr Glu Thr Val Arg Val Pro Gly
      60
Cys Ala His His Ala Asp Ser Leu Tyr Thr Tyr Pro Val
      70
Ala Thr Gln Cys His Cys Gly Lys Cys Asp Ser Asp Ser
      80
Thr Asp Cys Thr Val Arg Gly Leu Gly Pro Ser Tyr Cys
      90
Ser Phe Gly Glu Met Lys Glu
    
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as well as a novel protein comprising the known gonadotropin alpha-subunit and the 111 AA beta-subunit as here-above defined. The protein has follicle stimulating hormone (FSH) biological activity. Preferably it is in glycosylated form. Most preferably it is substantially free from detectable traces of luteinizing hormone and other urinary proteins.

A further aspect of the invention is a pharmaceutical composition containing a purified protein according to the present invention and a pharmaceutically acceptable excipient. Preferably the composition is in a form suitable for subcutaneous administration. It has been found that subcutaneous injection of a pharmaceutical composition according to the present invention does not give rise to the appearance of those allergic reactions normally encountered with the known FSH preparations.

According to a further aspect of this invention, a method is provided to produce the novel and highly purified FSH according to the present application. The method is substantially based on the combination of an immunopurification step with reverse phase HPLC. The immunopurification step uses immobilized monoclonal antibodies substantially as described in the above mentioned U.K. patent application 2,173,803A, but with the fundamental difference that elution of FSH from the immunocomplex is carried out at much higher pH values.

In the process of the present invention elution is conveniently carried out using an aqueous solution having a pH higher than about 10 and a molarity higher than about 0.5. The experiments carried out by Applicant have shown that, in these conditions the immobilized antibody is not substantially inactivated. This is in contrast with the teaching of the above mentioned U.K. patent application which states (on page 1, lines 36-37) that the use of eluents having such high pHs is not feasible in practice since it leads to rapid inactivation of the antibody.

This difference in behaviour may depend upon differences in the methods adopted to link the antibody to the resin.

According to this invention, the pH of the eluent is preferably higher than 11 and more preferably comprised within the range of 11.3 to 11.7.

Molarity values are preferably higher than 0.8 and more preferably of about 1.

Suitable eluents for use in the invention process are ammonia, diethylamine and such buffers as TRIS buffer, glycine-NaOH and the like.

The subsequent-reverse phase high pressure liquid chromatography (HPLC) step permits one to obtain the complete removal of any contaminating proteins. This removal is not achieved by the immuno-purification step alone, as is also acknowledged in the U.K. application text. The fact that such a step not only effectively removes the residual traces of contaminating proteins but also retains all the FSH

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biological activity is surprising in view of the article by P. Hallin and S. A. Khan (*J. Lig. Chromat.* 9(13), 2855-68, 1986) which shows loss of biological activity for bovine FSH and for human FSH (standard of HMG) when they are subjected to reverse-phase HPLC. On the other hand, the same article shows a satisfactory recovery of biological activity, but only partial separation, when the human urinary preparation (i.e., containing both FSH and LH) is subjected to ion-exchange HPLC.

Following the HPLC step, FSH is recovered using conventional techniques. For storage and/or handling purposes, the product can be lyophilized. Other suitable procedures to effect concentrating, stabilizing or other processing of the product are contemplated.

Clearly, the availability of post-menopausal urine is greater than that of human pituitary glands. This relative availability lends dramatic importance to Applicant's discovery.

The FSH-specific monoclonal antibody for use in the process of this invention may be produced using known techniques.

In a typical example, hybridoma cell line 9/14 was screened for optimal affinity towards FSH at the University of Cambridge. Monoclonal antibodies were produced in supernatant medium by cell culture techniques and purified by means of salt fractionation using 50% (w/v) ammonium sulphate followed by ionic reverse-phase chromatography and HPLC gel filtration and dialysis.

The monoclonal antibody produced by cell line 9/14 and purified as described above had the following specifications:

- a) protein purity: not less than 90%
- b) class of immunoglobulin: IgG 1
- c) affinity constant: $3.5 \times 10^8 \text{ L.mole}^{-1}$
- d) specificity:

Antigen	% cross reaction
FSH	100
HCG	1
HCG-beta subunit	1
LH	1
TSH	1

- e) binding capacity to FSH in solution: about 1,000 I.U. FSH per mg McAb.

FSH-specific monoclonal antibodies are chemically bound to Sepharose 4B by divinylsulphone according to the method described by J. Porath in *Methods in Enzymology* 34, pages 13-30, 1974.

In a typical example, 1 liter of Sepharose 4B was washed on a porous glass filter first with 5 liters of freshly distilled water and then with 5 liters of 1M sodium carbonate, pH 11. The washed Sepharose was suspended in 1 liter of 1 M sodium carbonate (pH 11). 200 ml of divinylsulphone were added dropwise with continuous stirring. The reaction was completed in 70 minutes at room temperature and then stopped by means of the addition of 1N HCl (up to pH 7.0).

The activated resin was suspended in 1 liter of 0.25M sodium bicarbonate (pH 9.0) containing 2 grams of anti-FSH monoclonal antibody to obtain more than 90% binding of the antibody to the activated resin. The immuno-resin obtained was stored at 4° C.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a comparison of highly purified urinary FSH (uFSH) with highly purified pituitary FSH (pFSH) on SDS-Page. Molecular weight markers (M.W.) are reported in the left lane.

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FIG. 2 is an analysis of highly purified urinary FSH (uFSH) and highly purified pituitary FSH (pFSH) by size exclusion chromatography. Retention times are reported at the top of the peak.

FIG. 3 is a comparison of highly purified urinary FSH (uFSH) and highly purified pituitary FSH (pFSH) by isoelectric focusing. Isoelectric point markers (I.E.F.) are reported in the left lane.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

The following Example 1 illustrates the two purification steps of the method of this invention as applied to commercial HMG, i.e., the active ingredient in Pergonal® Serrono.

It is understood that while HMG is the preferred starting material in the invention process, the latter is also applicable to less purified materials such as post-menopausal urine concentrates and the like. Good results have been obtained using as starting material a urinary concentrate containing as low as 1 I.U. FSH per mg.

Other aspects and advantages of the invention will become apparent after consideration of the following examples, which are not intended to be limiting.

EXAMPLE 1

Preparation of highly purified urinary FSH (hpuFSH)

1st Step: Immunopurification on anti FSH McAb-DVS-Sepharose

HMG was used as starting material.

Immuno-resin anti-FSH McAb-DVS-Sepharose was equilibrated in 0.1M Tris-HCl, 0.3M NaCl buffer pH=7.5 at 4° C.

The column was loaded with a quantity of IU FSH (RIA) corresponding to 80-90% of its total FSH binding capacity. The non-retained proteins were eluted with the equilibrating buffer until the OD₂₈₀ of eluate was lower than 0.02.

The absorbed uFSH was eluted from the immuno-resin with 1M ammonia solution at 4° C. Ammonia eluates corresponding to about 4 times the immuno-resin volume were pooled, the pH was adjusted to 9.0 as soon as possible at 4° C. by adding glacial acetic acid and the solution was ultrafiltered in an Amicon apparatus (membrane C.O. 10,000 Ds) and concentrated to a small volume.

2nd Step: Reverse phase HPLC

The resultant solution, adjusted at pH=5.6, was loaded on a C₁₈ reversed phase column (Pre Pak Waters) which had previously been equilibrated with 0.05M ammonium acetate pH=5.6 buffer at room temperature.

Flow rate was 100 ml/min and the eluate was monitored at 280 nm.

The HPLC purification was carried out employing a Prep 500 A apparatus (Waters) equipped with UV detector and a preparative gradient generator.

Biologically active highly purified urinary FSH was eluted by a gradient of isopropanol up to 50% of the mobile phase. Fractions were checked by analytical GPC and RIA.

The organic solvent was removed by distillation under vacuum at 40° C. and then the solution was frozen and lyophilized.

EXAMPLE 2

Characterization of the FSH

The highly purified urinary FSH preparation of the present invention was subjected to several chemico-physical, biological and immunological tests in order to achieve its complete characterization. As indicated below, most of the

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tests were carried out in comparison with a highly purified pituitary FSH preparation obtained according to the procedure described below.

Crude human pituitary extract (HPG), a by-product resulting from extraction of the Human Growth Hormone, was used as starting material.

The procedure consisted of the following steps:

- 1) Preliminary purification of crude HPG by extraction with 40% ethanol containing 6% ammonium acetate pH 5.6 and precipitation of supernatant with 96% cold ethanol.
- 2) Further purification of HPG by ion exchange chromatography on DEAE cellulose, using 0.15M sodium acetate pH 7 as eluent, followed by precipitation with 96% cold ethanol.
- 3) Further purification of FSH fraction by gel filtration on Sephadex G-100, using 0.05M ammonium bicarbonate as buffer.
- 4) Last step of FSH purification by ion exchange chromatography on SP Sephadex C 50. Highly purified pituitary FSH was eluted with 0.1M phosphate buffer pH 6.2 and lyophilized.

The pituitary FSH in-vivo biological specific activity (Steelman-Pohley test; LU. of 2nd IRP-HMG) was determined to be 4770 LU. FSH/mg of lyophilized powder using the methodology described below under "Biological Assay".

The characterization tests performed were as follows:

LH contamination

This test was carried out by means of a specific radioimmunoassay for LH using an LH standard calibrated against the 2nd IRP-HMG and 125 I-LH as the tracer, both contained in the LH ter KIT (code 10204) currently marketed by the company Biodata®. As antiserum, a sheep anti-HCG antiserum prepared by the Applicant was used having 100% cross-reactivity to LH and less than 0.1% to FSH.

The RIA procedure as per the manufacturer's instructions was followed to obtain as a result no detectable LH contamination in the highly purified urinary FSH preparation prepared in accordance with the Example 1. The minimum detectability of the LH ter KIT is 1.5 mIU/ml.

The above results were confirmed using a more sensitive assay called DELFIA (dissociation-enhanced lanthanide fluoro immunoassay) marketed by LKB-Pharmacia.

SDS—PAGE

Slab-gel electrophoresis in SDS was performed, under reducing conditions, on 13.5% polyacrylamide gel pH=8.8 according to the procedure described by Laemmli in *Nature*, 227, pages 680-685 (1970).

Staining was performed with Coomassie Brilliant Blue R-250 0.25% in a 25% methanol/75% acetic acid solution.

Both the hpuFSH preparation according to the invention and the highly purified pituitary FSH preparation (hppFSH) obtained as described above were subjected to this test. Results are illustrated in FIG. 1 where the bands of both the urinary and pituitary FSH preparations (uFSH and pFSH, respectively) are shown together with the molecular weight markers (M.W.).

In both the uFSH and pFSH preparations the main large band typical of the glycoproteins is found at the same molecular weight of approximately 20,000 Daltons, which is also in accordance with the literature data. Due to the known behaviour of the glycoproteins in SDS—PAGE, this value is a little overestimated.

Size exclusion chromatography

Analyses of both hpuFSH and hppFSH were performed on a TSK G 2000 SW column by LKB using 0.1M phosphate buffer pH 6.8+0.2M NaCl as the eluent. Flow was 0.7 ml/minute and readings were made at the wavelength of 230 nanometers.

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Results are illustrated in FIG. 2 which shows single main peaks for both uFSH and pFSH with substantially the same retention times (13.37 and 13.55 minutes, respectively).

Isoelectrofocusing

Isoelectrofocusing was carried out on 5% thin-layer polyacrylamide gel (Anpholine LKB®, pH range 3.5 to 9.5) fixed in 11.5% (w/v) TCA and 3.5% (w/v) sulphosalicylic acid and stained with a Silver staining BIO-RAD® Kit.

Results for both hppFSH and hpuFSH are illustrated in FIG. 3 which also shows the isoelectric point markers. As can be seen from the figure, both urinary and pituitary FSHs show several bands but completely different patterns; in particular, the main bands of urinary FSH are at a pH range lower than 4.8 whereas pituitary FSH shows the main bands at a higher pH range.

This test demonstrates that urinary and pituitary FSHs are not identical molecules and that the differences reside at least in their carbohydrate moieties, if not in the protein moieties themselves.

Biological assay

The FSH in-vivo biological activity of hpuFSH as tested by the Steelman Pohley method (*Endocrinology* 53, pages 604-616, 1953) using as the reference material an HMG House Standard calibrated against the 2nd International Reference Preparation of HMG for bioassay. This assay is accepted by all major Pharmacopocias and health Authorities for the determination of the International Units (I.U.) of FSH biological activity.

The hpuFSH preparation obtained according to the Example above was determined to have a specific activity of 6200 LU. of FSH per mg of lyophilized powder, i.e., the highest specific activity ever described for an FSH urinary preparation.

Determination of the Amino Acid sequence

a) UFSH Subunit Separation

The separation of the uFSH subunits is performed using a Waters HPLC system equipped with a column Aquapore RP-300, 200x4.6 mm, 7 μ m. (Brownlee Labs).

The eluents are A=0.1% TFA (Trifluoroacetic acid, HPLC grade, Pierce), B=0.055% TFA in Acetonitrile. The flow rate is 1 ml/min at 35° C. and the initial condition is 15% B.

The gradient raised 40% B in 20 min.

The UV detector is set at 229 nm.

Usually in analytical runs 20 μ mol of a solution 0.5 mg/ml of hpuFSH and 0.5% TFA is injected.

Preincubation in TFA solution is not necessary.

The preparative separation is performed using the same column.

Good resolution is achieved by injecting 0.5 mg of hp human u FSH (batch n 032) dissolved in A buffer. Fractions are collected manually and dried down using a Speed Vac concentrator. Subunits are dissolved in water, analyzed by HPLC and stored at -20° C.

The beta subunit is eluted after about 10.5 min as a broad peak, the alpha subunit elutes at 15 min as a sharp peak.

The recognition of each peak as beta and alpha subunit respectively is accomplished by a specific RIA.

Usually the recovery of the beta subunit is around 50%, the alpha subunit recovery on the contrary is more than 90%.

b) Reduction and Carboxymethylation of the alpha and beta Subunits

Reduction of the subunits, separated as described in point a), is performed in 0.5 Tris, 0.1% EDTA, 6M

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Guanidine HCl pH 8.5 for 2 h at 37° C. using DTT (dithiothreitol, Serva) in 15 fold molar excess over cystein content.

Sodium iodoacetate (2.1 molar over DTT) is then added to the reaction mixture and left lying for 30 min in the dark.

To stop the reaction 1% mercaptoethanol and TFA is added.

The carboxymethylated subunits are purified by HPLC. The beta carboxymethylated subunit is poorly soluble in water so that the final yield is quite low.

c) Reduction, pyridylethylation and trypsin digestion of the beta subunit

Reduction of the beta subunit, separated as described in point a) was performed at room temperature for 2 hrs in the following way:

0.1 mg of beta subunit 0.5 ml of 0.5M Tris. 0.1% EDTA, 6M guanidine HCl pH 8.5 2.7 mg Dithiothreitol

The Pyridylethylation was performed adding 0.02 ml of 4 vinylpyridine to the previous solution.

The reaction takes 2 hrs at room temperature.

Finally, the reaction mixture was loaded onto a C4 Cartridge (Baker) equilibrated with 0.1% TFA.

The cartridge was washed with 0.1% TFA and the beta subunit pyridylethylated was eluted with 40%

Acetonitrile, 0.1% TFA. The eluted fraction was dried down and purified by HPLC as previously reported for the beta subunit.

The pyridylethylated beta subunit previously purified by HPLC was dissolved in 1% NH_4HCO_3 , pH 8.

The trypsin was added to the solution and the reaction was carried out for 1.5 hrs at 37° C.

The tryptic fractions were purified by HPLC using the same procedure previously described, except for the gradient from 0% to 55% in 30' and the UV detector, set at 214 nm.

The tryptic fractions were sequenced in the same way reported for the sequencing of the subunits (cf. point e)).

d) Sequencing of the alpha subunit

Several sequencing runs are performed using alpha subunit, carboxymethylated alpha subunit or integral highly purified human UFSH usually loading 5 nmoles or less into the protein sequencer (470 A, Applied Biosystem) and using the standard 03RPTH program delivered by Applied Biosystem or a special program where cycles which cleave a Pro or a Gly are substituted by the special cycle 03CPR0.

The first 45 residues starting from NH_2 terminal of the molecule were identified and the results confirm the amino acid sequence known from the literature (J. Biol. Chem. 250, 6735 (1975).

The Asn at position 52 and at position 78 referring to the known sequence starting with Ala are the amino acids where glycosylation occurs.

At the NH_2 terminal is always present an heterogeneity and is always the same in all samples sequenced. The complete molecule (i.e. starting with Ala) is present in 65% of the chains, the molecule missing the first two amino acids (i.e. starting with Asp) is present in 5%, the molecule missing the first three amino acids (i.e. starting with Val) is present in 30%.

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	1			5		
65% of chains	Ala	Pro	Asp	Val	Gln	...
5% of chains			Asp	Val	Gln	...
30% of chains				Val	Gln	...

e) Sequencing of the beta subunit

Several sequencing runs were performed using beta subunit, carboxymethylated beta subunits, integral highly purified human u-PSH and tryptic peptides from pyridylethylated beta subunit.

The samples were loaded into the protein sequencer (470 A, Applied Biosystem) using the standard 03RPTH program delivered by Applied Biosystem. The results show that that beta-subunit is 111 amino acids long and has the following amino acid sequence:

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Asn	Ser	Cys	Glu	Leu	Thr	Asn ²⁰	Ile	Thr	Ile	Ala	Ile	Glu
Lys	Glu	Glu	Cys ³⁰	Arg	Phe	Cys	Ile	Ser	Ile	Asn	Thr	Thr
Trp ⁴⁰	Cys	Ala	Gly	Tyr	Cys	Tyr	Thr	Arg	Asp	Leu ⁵⁰	Val	Tyr
Lys	Asp	Pro	Ala	Arg	Pro	Lys	Ile ⁶⁰	Gln	Lys	Thr	Cys	Thr
Phe	Lys	Glu	Leu	Val ⁷⁰	Tyr	Glu	Thr	Val	Arg	Val	Pro	Gly
Cys	Ala ⁸⁰	His	His	Ala	Asp	Ser	Leu	Tyr	Thr	Tyr	Pro ⁹⁰	Val
Ala	Thr	Gln	Cys	His	Cys	Gly	Lys	Cys ¹⁰⁰	Asp	Ser	Asp	Ser
Thr	Asp	Cys	Thr	Val	Arg ¹¹⁰	Gly	Leu	Gly	Pro	Ser	Tyr	Cys
Ser	Phe	Gly	Glu	Met	Lys	Glu						

The Asn at position 7 and 24 referring to the above sequence starting with Asn at position 1 are the amino acids where glycosylation occurs. At the NH_2 terminal of beta subunit is always present an heterogeneity and is always the same in all samples sequenced. The complete molecule (i.e. starting with Asn) is present in 35% of the chains, the molecule missing the first amino acids (i.e. starting with Ser) is present in 15%, the molecule missing the first two amino acids (i.e. starting with Cys) is present in 50%.

	1			5		
35% of chains	Asn	Ser	Cys	Gly	Leu	...
15% of chains		Ser	Cys	Glu	Leu	...
50% of chains			Cys	Gly	Leu	...

Heterogeneity of beta subunit

EXAMPLE 3

Pharmaceutical preparations

The production of pharmaceutical dosage form preparations containing hpuFSH obtained in accordance with this invention is not particularly difficult. Since the substance maintains its biological activity after lyophilization, this is the preferred form for injectable preparations.

The lyophilized hpuFSH-containing preparation is reconstituted using physiological saline and/or other suitable diluents to yield an injectable solution.

Some excipients may be suitably used in the composition as a part thereof, such as, for example, mannitol, lactose, glycine, glucose, saccharose and their mixtures. Other conventional carriers, fillers and the like can be used. Mixtures are operable.

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In the experiments carried out according to the present invention, lactose has been used as the excipient for the injectable preparations.

In preparing injectable formulations, pH is optionally adjusted via the additional conventional acidic or basic substances. Acidic substances include acetic acid and the like. Basic substances include sodium hydroxide and the like. Mixtures can be employed.

The use of one or more stabilizers, e.g. albumin and the like, is contemplated.

A typical example of pharmaceutical production for the manufacturing of a batch of 20,000 ampoules each containing 75 LU. FSH is as follows.

The calculated (in units of biological activity) amount of the lyophilized FSH bulk powder is dissolved in 700 ml of cold apyrogenic water for injection. If necessary, pH is readjusted to a value between 6.2 and 6.8 by using either acetic acid or sodium hydroxide, as the case may be. The solution is then sterile filtered through a 0.2 micron pores filter.

200 grams of lactose are dissolved in 2 liters of apyrogenic water for injection, sterile filtered as above and added to the FSH solution.

Apyrogenic water for injection is added to reach a final volume of 15 liters, the solution is dispensed into ampoules (0.75 ml each) and lyophilized in a sterile lyophilizator.

Ampoules are obtained which contain each 75 LU. FSH and 10 mg lactose.

In a preferred embodiment the ampoules may contain 150 LU. FSH.

According to a further example of pharmaceutical preparation, ampoules have been prepared which also contain 1 mg of human albumin as stabilizer in addition to the excipient lactose.

Although this invention has been illustrated with specific examples, it is understood that variations may be made without departing from the spirit and scope of the invention.

We claim:

1. A substantially pure biologically active follicle stimulating hormone preparation free from traces of luteinizing hormone detectable at 1.5 mIU/ml, based on the 2nd IRP-HMG reference standard for luteinizing hormone, and substantially free from all other urinary proteins, comprising an alpha-subunit associated with a beta-subunit,

wherein the alpha-subunit comprises a heterogeneous population of glycosylated human gonadotropin alpha-subunits having (1) the following amino acid sequence α :

```

10 Ala Pro Asp Val Gln Asp Cys Pro Gln Cys Thr
    Leu Gln Gln Asn Pro
20 Phe Phe Ser Gln Pro Gly Ala Pro Ile Leu Gln
    Cys Met Gly Cys Cys
40 Phe Ser Arg Ala Tyr Pro Thr Pro Leu Arg Ser
    Lys Lys Thr Met Leu
50 Val Gln Lys Asn Val Thr Ser Gln Ser Thr Cys

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-continued

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60 Cys Val Ala Lys Ser
70 Tyr Asn Arg Val Thr Val Met Gly Gly Phe Lys
80 Val Gln Asn His Thr
90 Ala Cys His Cys Ser Thr Cys Tyr Tyr His Lys Ser;
100 Arg Gly Leu Gly Pro Ser Tyr Cys Ser Phe Gly
110 Glu Met Lys Gln;

```

(2) the amino acid sequence α but lacking the starting Ala and Pro at positions 1 and 2; and (3) the amino acid sequence α but lacking the starting Ala, Pro and Asp at positions 1-3, and

wherein the beta-subunit comprises a heterogeneous population of glycosylated beta-subunits having (1) the following amino acid sequence β :

```

10 Asn Ser Cys Gln Leu Thr Asn Ile Thr Ile Ala
    Ile Gln Lys Gln Gln
20 Cys Arg Phe Cys Ile Ser Ile Asn Thr Thr Trp
30 Cys Ala Gly Tyr Cys
40 Tyr Thr Arg Asp Leu Val Tyr Lys Asp Pro Ala
    Arg Pro Lys Ile Gln
50 Lys Thr Cys Thr Phe Lys Gln Leu Val Tyr Gln
60 Thr Val Arg Val Pro
70 Gly Cys Ala His His Ala Asp Ser Leu Tyr Thr
80 Tyr Pro Val Ala Thr
90 Gln Cys His Cys Gly Lys Cys Asp Ser Asp Ser
    Thr Asp Cys Thr Val
100 Arg Gly Leu Gly Pro Ser Tyr Cys Ser Phe Gly
110 Glu Met Lys Gln;

```

(2) the amino acid sequence β but lacking the starting Asn at position 1; and (3) the amino acid sequence β but lacking the starting Asn and Ser at positions 1 and 2.

2. A preparation in accordance with claim 1, wherein the Asn at positions 7 and 24 of said beta-subunit and the Asn at positions 52 and 78 of said alpha-subunit are glycosylated.

3. A preparation in accordance with claim 1, having a specific activity when in the form of a lyophilized powder of about 6200 International units of follicle stimulating hormone per milligram of lyophilized powder.

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4. A pharmaceutical composition for treating infertility containing a therapeutically effective amount of a preparation in accordance with claim 1, and a pharmaceutically acceptable excipient.

5. A pharmaceutical composition in accordance with claim 4, wherein said excipient is lactose.

6. A pharmaceutical composition in accordance with claim 4, in unit dosage form, which contains about 75 International units of follicle stimulating hormone per unit dose.

7. A pharmaceutical composition in accordance with claim 4, in unit dosage form, which contains about 150

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International units of follicle stimulating hormone per unit dose.

8. A pharmaceutical composition in accordance with claim 4, further including human albumin as stabilizer.

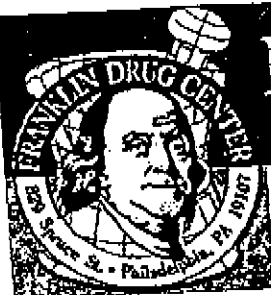
9. A preparation in accordance with claim 1, having a specific activity when in the form of a lyophilized powder of 6200 International units of follicle stimulating hormone per milligram of lyophilized powder.

10. A pharmaceutical composition in accordance with claim 7, further including about 1 mg human albumin as stabilizer.

* * * * *

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FDA APPROVES BRAVELLE™ (urofollitropin injection, purified)

FERRING'S NEW HUMAN-DERIVED FOLLICLE-STIMULATING HORMONE
INFERTILITY TREATMENT



New Highly Purified Alternative To Genetically Engineered Infertility Treatments

TARRYTOWN, NY – May 6, 2002 – Ferring Pharmaceuticals, a world leader in occurring protein hormones, announced today that it has received approval from the Food and Drug Administration (FDA) to market Bravelle™ (urofollitropin for injection, purified), a highly purified, human-derived follicle-stimulating hormone (hFSH) for treatment of infertility. Bravelle™, in conjunction with human chorionic gonadotropin (hCG), is indicated for ovulation induction following pituitary suppression.

"With the introduction of Bravelle™, Ferring has expanded its family of human-derived hormones to include a highly purified, well-tolerated hFSH with proven efficacy for ovulation induction, a critical step in many infertility treatment protocols," said Dr. Robert Anderson, president of Ferring Pharmaceuticals.

"Based on the fact that recombinant technology has shown no meaningful advantage in either efficacy or safety in the clinic, Ferring remains committed to the development of human-derived products in order to seek improvements in ovarian stimulation. Ferring has submitted an application to the FDA seeking additional indication for Bravelle™ in infertility treatment. This application, which is supported by additional clinical data, is currently under review."

FPI007745

clinical studies, brings the total number of patients studied to 577. This application is currently under review by the FDA."

A Human-Derived FSH Proven as Safe and Effective as Genetically Engineered FSH
Bravelle™ was compared to follitropin beta, a recombinant FSH, in a prospective, multicenter trial in 111 oligo-anovulatory patients undergoing ovulation induction. Patients underwent pituitary suppression with a GnRH agonist prior to being treated with Bravelle™ SC, Bravelle™ IM or follitropin beta SC. Results showed that there were no significant differences in efficacy and safety between the treatment groups.

Percentage of patients Bravelle™ SC Follitropin beta SC

achieving:	(n=26)	(N=35)
Ovulation	96.1%	85.7%
Clinical pregnancy	34.6%	31.4%
Continuing pregnancy	34.6%	28.6%
Live birth	34.6%	17.1%

In addition to the studies supporting the new drug application, Ferring has recently completed two Phase 3B clinical trials involving 24 centers. These trials evaluated Bravelle™ together with Repronex® (mixed protocol), Ferring's human chorionic gonadotropin, in the same syringe, in two age groups. The first study evaluated the mixed protocol in 108 women ages 18 to 33 years; the second trial evaluated the mixed protocol in 108 women ages 34 to 40 years. This is the first time a prospective, systematic clinical evaluation of single daily dose mixed protocols has been conducted anywhere in the world.

Bravelle™: The Natural Choice

Bravelle™ is affordably priced, an important benefit since infertility treatments are not fully covered by insurance. It is available for both subcutaneous and intramuscular injection. Most patients prefer SC administration because it is more convenient and causes less discomfort.

Added Anderson, "Bravelle™ is ideally suited to meet the needs of infertility patients by providing an affordable solution that combines human-derived efficacy with recombinant hormone-like purity."

Only physicians thoroughly familiar with infertility treatment, including their patients' medical histories and adverse reactions, should prescribe Bravelle™. Like all gonadotropins, Bravelle™ is a potent substance capable of causing mild to severe adverse reactions including ovarian hyperstimulation syndrome (incidence of 8.2%), with or without pulmonary or vascular complications, in women undergoing therapy for infertility.

Background on Human-Derived Hormones

The key differences in human-derived and genetically engineered infertility treatments are raw material sources and cost. Human-derived FSH treatments are highly purified FSHs extracted from the urine of postmenopausal women. By comparison, genetically engineered products are derived from the secretions from Chinese hamster oocytes, which are cultured in fetal calf or other mammalian serum, and approximate human FSH.

FPI007746

Bravelle™ For Infertility - Franklin Drug Center

Both are manufactured in compliance with extremely strict standards (including inactivation and confirmatory testing), but human-derived products are generally expensive than their genetically engineered counterparts.

About Ferring

Ferring Pharmaceuticals, part of the Ferring Group, a privately owned, international pharmaceutical company, markets Bravelle™, Repronex® and Novarel® in the United States. Ferring Group specializes in the development and commercialization of compounds in general and pediatric endocrinology, gastroenterology, obstetrics/gynecology and infertility.

For more information, call 1-888-337-7464 or visit www.ferringusa.com.

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Regards

Ronald S. Cohen, BS Ph.
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Mixed-Protocol, Same-Syringe Combination of Gonadotropins: Compatibility of New, Highly Purified, Human-Derived FSH (Bravelle™) and hMG (Repronex®)

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Key words: Bioactivity, Bravelle, Repronex, urofollitropin, menotropins, reconstitution, mixed preparation, same syringe.

Abstract

Objective. To determine if LH and FSH bioactivities were altered by reconstituting Ferring Pharmaceuticals' new, highly purified human-derived FSH (Bravelle™) in 0.9% saline and mixing with Ferring's hMG (Repronex®) in the same syringe.

Design. The FSH and LH bioactivities were determined after injecting 21-day-old in-bred Wistar female and Sprague-Dawley male rats with a mixture of Bravelle™ and Repronex® (150:75 IU, FSH:LH). Ovarian weights (FSH bioactivity) or seminal vesicle weights (LH bioactivity) were recorded. The bioactivities of FSH and LH were compared to a Reference Standard.

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FPI002721

Intervention(s). A menotropin Reference Standard was diluted in a Reference Standard diluent to a low, middle and high dose. Bravelle™ was reconstituted in 0.9% saline and mixed with Repronex® in a Becton-Dickinson plastic syringe. The mixture (analyte) was subdivided with the Reference Standard diluent to provide the same 3 doses as the Reference Standard. The study animals were injected with the assigned solution.

Main Outcome Measure(s). Ovarian weights and seminal vesicle weights.

Results. The theoretical bioactivity of FSH in the analyte was 169.21 IU/vial, and the actual bioactivity was 172.37 IU/vial. The theoretical bioactivity of LH in the analyte was 83.13 IU/vial and the actual bioactivity was 84.64 IU/vial.

Conclusion. Mixing Ferring's human-derived FSH (Bravelle™) with Ferring's hMG (Repronex®) does not result in a decrease or increase in the bioactivity of either FSH or LH.

Introduction

In 1942, Greep and co-workers¹ were the first to show that follicle-stimulating hormone (FSH) and luteinizing hormone (LH) were distinct chemical moieties, and that both FSH and LH were necessary for follicular growth, ovulation and formation of the corpus luteum. The first successful use of gonadotropins to induce ovulation in anovulatory women was reported 16 years after that discovery.² Since then, gonadotropins have been widely used to treat infertile women with oligomenorrhea and amenorrhea that is not secondary to ovarian failure. In addition, gonadotropins are extensively used to stimulate multiple follicular development in women undergoing assisted reproduction.³

The first major commercially available human menopausal gonadotropin (hMG) was purified from the urine of postmenopausal women, and contains equal amounts of FSH and LH. Subsequently, FSH was prepared from postmenopausal urine by absorbing LH using anti-hCG (human chorionic gonadotropin) antibodies and filtration

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With the emergence of biotechnological methods, recombinant FSH produced *in vitro* by genetically engineered Chinese hamster ovary (CHO) cells became available for clinical use.⁷ Recombinant FSH is prepared by transfecting CHO cells with FSH- α and - β subunits. The amino acid sequence of recombinant FSH is identical to human FSH, although glycosylation of FSH is carried out by the CHO cells.⁸ A major difference between recombinant and human-derived FSH preparations that might have implications for efficacy is the lack of LH activity of recombinant FSH. In hypogonadotropic-hypogonadal women, recombinant FSH induced normal follicular growth. However, estrogen production was suboptimal.⁹

When a gonadotropin-releasing hormone (GnRH) antagonist was used to suppress the pituitary in women undergoing ovarian stimulation with recombinant FSH, endogenous LH levels fell with increasing doses of the antagonist. Serum E₂ levels fell when LH levels decreased. However, the mean number of follicles was similar across all doses of the antagonist.¹⁰ These results suggest that although FSH by itself can induce follicle development, LH may be required for follicle maturation. The amount of LH necessary for normal follicle development is not known. Filicori et al.¹¹ treated patients who were infertile or anovulatory with either FSH alone or FSH supplemented with 50 IU/day of hCG subcutaneously. They showed that the development of large follicles was enhanced and the duration of FSH treatment and the dose of FSH were reduced in patients who received hCG supplementation.

Most *in vitro* fertilization (IVF) centers utilize controlled ovarian hyperstimulation (COH) for multiple follicular development. Although present data suggest that FSH in combination with hMG is at least comparable to hMG or FSH alone for COH,¹² clinicians often combine FSH and hMG in their stimulation protocols to enhance the recruitment and maturation of follicles. For patient convenience and comfort, physicians often reconstitute these differing hormonal preparations with different diluents, sometimes from different manu-

for Bravelle[®] and Repronex[®] across all assays.

Test Animals

In-bred Wistar female rats (n=78) at 21 days of age were used for the FSH bioassay, and Sprague-Dawley male rats (n=104) at 21 days of age were used for the LH bioassay. The test animals were certified to be free from murine viruses. Upon arrival, the animals were weighed,

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their health was assessed, and they were randomized into groups of 6 (females) or 8 (males). The animals were housed in polypropylene solid bottom cages with stainless steel wire lids containing shredded Aspen shavings. The animal room was maintained at 18-19°C, with relative humidity of approximately 50% and a 12:12 hour, light:dark cycle. Purina rodent diet was given along with tap water *ad libitum*.

For each assay (FSH or LH), 3 groups of study animals were assigned in duplicate to the Reference Standard: low-, middle-, and high-dose, and 3 groups were assigned in duplicate to the analyte (i.e., mixture of Bravelle™ and Repronex®): low-, middle- and high-dose. One group of rats was assigned to the control solution.

FSH Assay

Reference Standard. Menotropin Reference Standard (Ferring, lot DPH12250397, 122.2 FSH IU/mg, traceable to the NIBSC) was dissolved in a diluent that contained 10.75 g disodium hydrogen phosphate, 7.6 g sodium chloride, 1.0 g bovine serum albumin, and 70,000 units hCG/liter of distilled water. The pH of the solution was adjusted to 7.2±0.2 with 1 N sodium hydroxide. The solution was then subdivided to 3 concentrations: 2, 4 and 8 IU/0.6 ml, which were established in a geometric progression for a low, middle and high dose, respectively, by previous dose-response studies. The lowest dose in this 3-dose range produces a definite response in some of the test animals as compared to a control group, and the highest dose produces a submaximal to maximal response.

Analyte (Mixture of Bravelle™ and Repronex®). As the diluent, 6 vials of Bravelle™ (lot FMA001) were each reconstituted with 1 ml of 0.9% saline (supplied by the manufacturer) and then pooled. As the analyte, 5 vials of Repronex® (lot 464-891) were each reconstituted with this diluent and pooled. Reconstitutions and pooling were done with commercially available Becton-Dickinson (BD) plastic syringes. The final solution was subdivided to the same 3 concentrations as for the Reference Standard (i.e., 2, 4 and 8 IU/0.6 ml), using the Reference Standard diluent.

Control Solution. The Reference Standard diluent was used as the control solution.

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Procedure. Each female rat was injected subcutaneously with 0.2 ml of its assigned solution. The injections were given at approximately the same time of day for 3 consecutive days. Twenty-four hours after the last injection, the rats were sacrificed in a carbon dioxide chamber. The left and right ovaries were carefully dissected out from each animal and freed from any fat or fibrous tissue. The ovaries were dried by gentle blotting on absorbent paper and weighed on an analytical balance. The combined ovarian weights were recorded for each rat.

LH Assay

Reference Standard. The menotropin Reference Standard (Ferring, lot DPH12250397, 99.3 LH IU/mg, traceable to the NIBSC) was dissolved as described for the FSH assay, except that the diluent did not contain hCG. The solution was then subdiluted to 3 concentrations: 7, 14, and 28 IU/0.8 ml, which were established in a geometric progression for a low, middle and high dose, respectively, by previous dose-response studies. The lowest dose in this 3-dose range produces a definite response in some of the test animals as compared to a control group, and the highest dose produces a submaximal to maximal response.

Analyte (Mixture of Bravelle™ and Repronex®). As the diluent, 20 vials of Bravelle™ (lot FMA001) were each reconstituted with 1 ml of 0.9% saline (supplied by the manufacturer) and then pooled. As the analyte, 14 vials of Repronex® (lot 464-891) were each reconstituted with this diluent and pooled. Reconstitutions and pooling were done with BD plastic syringes. The solution was subdiluted to obtain the same 3 concentrations as the Reference Standard (i.e., 7, 14 and 28 IU/0.8 ml), using the Reference Standard diluent.

Control Solution. The Reference Standard diluent was used as the control solution.

Procedure. Each male rat was injected with 0.2 ml of its assigned solution. The injections were given at approximately the same time of day for 4 consecutive days. Twenty-four hours after the last injection, the rats were sacrificed in a carbon dioxide chamber. The seminal vesicles were carefully dissected out from each animal, dried by gentle blotting on absorbent paper and weighed on an analytical balance.

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Determination of FSH and LH Bioactivity (Potency Calculations and Acceptance or Rejection of Potency Values)

Both the FSH and LH assays are 3x3 parallel line assays performed in duplicate, where the responses to 3 doses of the analyte are compared to the responses to the same 3 doses of the Reference Standard. The result from each replicate is combined for the final result. Briefly, for each replicate, the ovarian weights or the seminal vesicle weights were used to calculate potency values for FSH and LH, respectively. For potency values to be accepted, the combined L-value (i.e., the confidence limit) from the duplicate assays has to be <0.18 and the range of the actual claim must be between 80% and 125%. If these specifications are not met, the bioassay has to be repeated until the combined L-value is <0.18.

Potency calculations were similar for FSH and LH and the following equations were used: ($M' = ciT/T_s$; where M' = log-potency of an unknown relative to its assumed potency; $c = 4/3$; i = interval in logarithms between successive log-doses, the same for standard and unknown; T_s = difference in the responses to the standard and to the unknown; and T_s = combined slope of the dosage-response curves for standard and unknown). After calculating M' , the log-potency was determined by the equation: $M = M' + \log R$ (M = log-potency; for the present experiment: $R=1$ making $\log R=0$). Therefore, potency=antilog M , and % claim=antilog $M \times 100$.¹⁴ Bioactivities for FSH and LH in the mixed preparation were compared with the actual bioactivities (adjusted claim) of FSH and LH in the starting preparations, rather than the bioactivities set forth in the product label (labeled claim).

Results

The bioactivity of FSH in Bravelle[®] from a previous assay was 82.40 IU/vial (adjusted claim), compared with a labeled claim of 75 IU/vial. The bioactivity of FSH in Repronex[®], also from a previous assay, was 86.81 IU/vial (adjusted claim), compared with a labeled claim of 75 IU/vial. Mixing the two hormones would therefore be expected to result in a combined FSH bioactivity of 169.21 IU/vial. The bioactivity of FSH in the mixed preparation from this study was 172.37 IU/vial. Since the combined L-value from the duplicate assays

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was 0.1, and therefore less than the specified USP L-value of 0.18, the actual FSH bioactivity of the mixed preparation determined by the bioassay was accepted.[†] Additionally, the range of bioactivity according to USP specifications must be between 80% (low range) and 125% (high range) of the actual claim. This requirement was also met (Table 1). Since all of the required USP specifications for the FSH bioassay were met, it can be concluded that the bioactivity of FSH in Bravelle[™] and Repronex[®] is unaffected by reconstituting Bravelle[™] in 0.9% saline and mixing with Repronex[®] in a BD plastic syringe.

The bioactivity of LH in Repronex[®] from a previous assay was 83.13 IU/vial (adjusted claim), compared with a labeled claim of 75 IU/vial. Mixing of Bravelle[™] with Repronex[®] would therefore be expected to result in LH bioactivity of 83.13 IU/vial. The bioactivity of LH in the mixed preparation from this study was 84.64 IU/vial. Since the combined L-value from the duplicate assays was 0.08, and therefore less than the specified USP L-value of 0.18, the actual LH bioactivity of the mixed preparation determined by the bioassay was

TABLE 1. Theoretical and actual FSH and LH bioactivities when Bravelle[™] is reconstituted in 0.9% saline and mixed with Repronex[®] (150:75 IU; FSH:LH) in the same syringe.

Hormone	Bravelle [™]	Repronex [®]	Theoretical bioactivity for the resulting mixture [*]	Actual bioactivity for the resulting mixture ^{**}	L-value [‡]
FSH bioactivity, IU/vial (% claim)	82.40	86.81	169.21 (100%)	172.37 (102%) (153.6-193.4) ^{††}	0.1
LH bioactivity, IU/vial (% claim)	0	83.13	83.13 (100%)	84.64 (102%) (77.0-93.0) ^{††}	0.08

* Obtained by combining the FSH or LH bioactivities for Bravelle[™] and Repronex[®] from separate bioassays.

** Obtained from the present bioassays.

† Low and high range.

‡ L-value refers to the confidence limit obtained after combining two replicates. L-value must be <0.18 for the bioassay results to be accepted.

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accepted." Additionally, the range of bioactivity according to USP specifications must be between 80% (low range) and 125% (high range) of the actual claim. This requirement was also met (Table I). Since all of the required USP specifications for the LH bioassay were met, it can be concluded that the bioactivity of LH in Repronex® is unaffected by reconstituting Bravelle™ in 0.9% saline and mixing with Repronex® in a BD plastic syringe.

Discussion

Gonadotropins are extensively used for treating infertile women with oligomenorrhea, and for stimulating multiple follicular development in women undergoing assisted reproduction.¹ An increasing number of physicians are combining hMG and FSH preparations in their stimulation protocols. For optimal patient convenience and comfort, various hormonal preparations, sometimes from different manufacturers (i.e., human-derived and recombinant) are often reconstituted with different diluents and mixed in a plastic syringe for administration to patients. Although this procedure reduces the number of injections, the authors are not aware of any previous studies investigating whether mixing of different gonadotropins affects the biological activities of FSH or LH.

The bioassay results reported in this study indicate that mixing Ferring's hMG (Repronex®) with Ferring's highly-purified, human-derived FSH (Bravelle™) does not decrease or increase the bioactivity of either FSH or LH. However, it must be noted that these results were obtained with FSH- and LH-containing preparations produced by a single manufacturer, and that the hormone preparations were reconstituted and mixed with well-defined and compatible diluents under carefully controlled conditions. Thus, the compatibility documented by the results of this study may not apply to other hormonal preparations or mixing conditions.

For instance, recombinant FSH preparations differ from human-derived FSH preparations with respect to oligosaccharide composition (glycosylation). The charge distribution (described by the isoelectric point) and the carbohydrate complexity (i.e., simple, intermediate and complex carbohydrates) were found to be quite different when recombinant FSH was compared with serum FSH throughout the menstrual cycle.¹⁰ Therefore, it is possible that when hormones

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with different chemical configurations are mixed, the compatibility and ultimately the bioactivity could be affected.

The activity of peptides may also be significantly affected by the diluents in which they are dissolved. In tests of preservative effectiveness, samples from two batches of a 1:1.5 dilution of epoetin alfa (4,000 units/ml) with bacteriostatic 0.9% sodium chloride for injection met the USP criteria for preserved solutions, whereas one of the two batches of a 1:1 dilution (5,000 units/ml) did not.¹¹ This result suggests that very subtle differences in dilution of proteins may significantly affect their biological activity. This conclusion is consistent with the view that different proteins may optimize internal packing and external solvent interactions by very different mechanisms.¹⁴

Mixture of proteins with other agents has the potential to affect their biological activity as well, and these interactions are also difficult to predict. Anderson and co-workers¹⁵ evaluated the effects on the activity of recombinant interleukin-2 of mixing with gentamicin sulfate, tobramycin sulfate, amikacin sulfate, ticarcillin disodium, piperacillin sodium, morphine sulfate, and total parenteral nutrient solution. Of all the agents evaluated, only ticarcillin disodium reduced the activity of recombinant interleukin-2.

The characteristics of the vessel (e.g., syringe) used for mixing or administering proteins such as FSH or LH can also affect the biological activity of the materials delivered to the patient. Results from a number of studies have shown that peptides or proteins may adhere to specific materials. Law and Shih¹⁶ reported that surface adsorption of calcitonin on soda lime silica glass is pH-dependent. Amiji and Park¹⁷ concluded that fibrinogen adheres to both dimethyldichlorosilane-treated glass and low-density polyethylene. To our knowledge, there have been no reports of loss of FSH or LH bioactivity with mixing or adhesion to vessel surfaces. Although the bioassay results of the present study support mixing of specific gonadotropin preparations from a single manufacturer, these findings cannot be extended to other situations, particularly the mixing of recombinant with human-derived gonadotropins.

In summary, the results of the bioassays from this study demonstrate that the Ferring gonadotropin products Bravelle[™] and Repronex[®] can be reconstituted with the diluent provided by this manufacturer (0.9% saline) and mixed in a commercially available BD plastic syringe without altering FSH or LH bioactivity. This determi-

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nation is very important because of the increasing number of physicians who are currently taking the approach of reconstituting and mixing, in the same syringe, gonadotropins from different manufacturers to treat infertile women. This practice raises the question of compatibility of different hormones when mixed and administered to patients. By selecting hMG and FSH preparations from the same manufacturer that have been tested for compatibility, and by reconstituting with the tested and approved manufacturer's diluent, both the health care provider and patient can be assured that the expected doses of FSH and LH are being delivered without alteration in the activity of either hormone.

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Bravelle™ (urofollitropin for injection, purified) for subcutaneous and intramuscular injection

BRAVELLE™

(urofollitropin for injection, purified) FOR SUBCUTANEOUS AND INTRAMUSCULAR INJECTION

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DESCRIPTION

Bravelle™ is a product containing a highly purified preparation of human follicle stimulating hormone (hFSH) extracted from the urine of postmenopausal women. Human FSH consists of two non-covalently linked glycoproteins designated as the α and β subunits. The α subunit has 92 amino acids of which two are modified by attachment of carbohydrates. The β subunit has 111 amino acids of which two are modified by attachment of carbohydrates.

Bravelle™ is a sterile, lyophilized powder intended for subcutaneous (SC) or intramuscular (IM) injection after reconstitution with sterile 0.9% Sodium Chloride Injection, USP. Each vial of Bravelle™ contains 82.5 International Units (IU) of Follicle Stimulating Hormone (FSH) activity, 23 mg Lactose Monohydrate, 0.005 mg Polysorbate 20, and Sodium Phosphate buffer (Sodium Phosphate dibasic, Heptahydrate and Phosphoric acid) for pH adjustments, which, when reconstituted with diluent, will deliver 75 IU of FSH. Bravelle™ contains up to 2% luteinizing hormone (LH) activity based on bioassay. Human Chorionic Gonadotropin (hCG) is not detected in Bravelle™. When stored at 3° to 25° C, up to 40% of the α -subunits may be oxidized.

The in vivo biological activity of urofollitropin for injection, purified is determined by using reference standards calibrated against the First International Standard for follicle-stimulating hormone, (FSH, Urofollitropin), Urinary, Human for Bioassay, National Institute for Biological Standards and Control (NIBSC) at its 46th meeting in 1995.

FSH is a glycoprotein that is acidic and water-soluble.
 Therapeutic class: Infertility.

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CLINICAL PHARMACOLOGY

Bravelle™ administered for 7 to 12 days produces ovarian follicular growth in women who do not have primary ovarian failure. Treatment with Bravelle™ in most instances results only in follicular growth and maturation. When sufficient follicular maturation has occurred, hCG must be given to induce ovulation.

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PHARMACOKINETICS

Single doses of 225 IU and multiple daily doses (7 days) of 150 IU of Bravelle™ were administered to healthy volunteer female subjects while their endogenous FSH was suppressed. Sixteen subjects received Bravelle™ SC and 12 received the drug IM. Serum FSH concentrations were determined. Based on the steady state ratio of

FSH C_{max} and AUC, SC and IM administration of Bravelle™ were not bioequivalent. Multiple doses of Bravelle™ IM resulted in C_{max} and AUC of 77.7% and 81.8% compared to multiple doses of Bravelle™ SC. The FSH pharmacokinetic parameters for single and multiple dose Bravelle™, administered SC and IM are in Table 1.

Table 1. FSH Pharmacokinetic Parameters Following Bravelle™ Administration.

PK Parameters	Single Dose (225 IU)		Multiple Dose X 7 (150 IU)	
	SC	IM	SC	IM
C_{max} (mIU/mL)	6.0 (1.7)	8.8 (4.5)	14.8 (2.9)	11.5 (2.9)
T_{max} (hrs)	20.5 (7.7)	17.4 (12.2)	9.6 (2.1)	11.3 (8.4)
AUC _{obs} (mIU • hr/mL)	379 (111)	331 (179)	234.7 (77.0)	192.1 (52.3)
$t_{1/2}$ (hrs)	31.8	37	20.6	15.2
Ka (hr ⁻¹)	0.0500 (0.0231)	0.1408 (0.1227)	0.0905 (0.0393)	0.0358 (0.0108)

Absorption

The maximum plasma concentration of FSH was attained at 20.5 and 17.4 hours following SC and IM single dose administration, respectively. However, following multiple dosing, it was attained at approximately 10 hours following both routes of administration.

Distribution

Human tissue or organ distribution of FSH has not been studied for Bravelle™.

Metabolism

Metabolism of FSH has not been studied for Bravelle™ in humans.

Elimination

The mean elimination half-lives of FSH for SC and IM single dosing are 31.8 and 37 hours, respectively. However, following multiple dosing (X 7 days) they are 20.6 and 15.2 hours for SC and IM, respectively.

Pediatric Populations

Bravelle™ is not indicated in pediatric populations.

Geriatric Populations

Bravelle™ is not indicated in geriatric populations.

Special Populations

The safety and efficacy of Bravelle™ in renal and hepatic insufficiency have not been studied.

Drug Interactions

No drug/drug interaction studies have been conducted for Bravelle™ in humans.

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CLINICAL STUDIES

The efficacy and safety of Bravelle™ was established in two randomized, active controlled, multi-center studies, one for in-vitro fertilization [IVF] and one for ovulation induction [OI].

Ovulation Induction

In the randomized, controlled ovulation induction study, patients underwent pituitary suppression with a GnRH

agonist before being randomized to Bravelle™ SC, Bravelle™ IM or a commercial recombinant FSH product administered SC. A total of 111 oligo-anovulatory patients were randomized of whom 72 received Bravelle™, starting at a dose of 150 IU daily for 5 days. This was followed by individual titration of the dose from 75 to 450 IU daily based on ultrasound and estradiol (E₂) levels. The total duration of dosing did not exceed 12 days. Results for the Intent To Treat Population are summarized in Table 2.

Table 2. Efficacy Outcome by Treatment Groups in Ovulation Induction for Study FPI FSH 99-03 (one cycle of treatment)

	Bravelle™ SC	Bravelle™ IM
Parameter	N=35	N=37
Ovulation (%)	24 (68.6)	26 (70.3)
Received hCG (%)	25 (71.4)	28 (75.7)
Mean Peak Serum E ₂ (pg/mL) (SD)	976.5 (680.6)	893.2 (815.2)
Chemical Pregnancy (%)	11 (31.4)	8 (21.6)
Clinical Pregnancy (%)	9 (25.7)	7 (18.9)
Continuing Pregnancy (%)	9 (25.7)	7 (18.9)
Pts. w/Live Births (%)	9 (25.7)	6 (16.2)

Assisted Reproductive Technologies [ART]

In the randomized, controlled IVF study FPI FSH 2001-01, patients underwent pituitary suppression with a GnRH agonist before being randomized to Bravelle™ SC or a commercial recombinant FSH product administered SC. A total of 120 patients were randomized of whom 60 received Bravelle™, starting at a dose of 225 IU daily for 5 days. This was followed by individual titration of the dose from 75 to 450 IU daily based on ultrasound and estradiol (E₂) levels. The total duration of dosing did not exceed 12 days. Results are summarized in Table 3 for the Intent-To-Treat population.

Table 3. Efficacy Outcome for IVF Study FPI FSH 2001-01 (one cycle of treatment)

	Bravelle™ SC
Parameter	n=60
Mean Total Oocytes Retrieved Per Patient (SD)	11.8 (6.3)
Mean Mature Oocytes Retrieved Per Patient (SD)	9.0 (5.7)
Patients with Oocyte Retrieval (%)	57 (95.0)
Patients with Embryo Transfer (%)	57 (95.0)
Patients with Chemical Pregnancy (%)	28 (46.6)
Patients with Clinical Pregnancy (%)	25 (41.7)
Patients with Continuing Pregnancy (%)	23 (38.3)

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INDICATIONS AND USAGE

Ovulation Induction

Bravelle™ administered SC or IM in conjunction with hCG, is indicated for ovulation induction in patients who

have previously received pituitary suppression.

Multifollicular Development during ART

Bravelle™ administered SC in conjunction with hCG is indicated for multiple follicular development (controlled ovarian stimulation) during ART cycles in patients who have previously received pituitary suppression.

Selection of Patients

1. Before treatment with Bravelle™ is instituted, a thorough gynecologic and endocrinologic evaluation must be performed. Except for those patients enrolled in an *in vitro* fertilization program, this should include a hysterosalpingography (to rule out uterine and tubal pathology) and documentation of anovulation by means of basal body temperature, serial vaginal smears, examination of cervical mucus, determination of serum (or urine) progesterone, urinary pregnanediol and endometrial biopsy. Patients with tubal pathology should receive Bravelle™ only if enrolled in an *in vitro* fertilization program.
2. Primary ovarian failure should be excluded by the determination of gonadotropin levels.
3. Careful examination should be made to rule out the presence of an early pregnancy.
4. Patients in late reproductive life have a greater predilection to endometrial carcinoma as well as a higher incidence of anovulatory disorders. Cervical dilation and curettage should always be done for diagnosis before starting Bravelle™ therapy in such patients who demonstrate abnormal uterine bleeding or other signs of endometrial abnormalities.
5. Evaluation of the husband's fertility potential should be included in the workup.

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CONTRAINDICATIONS

Bravelle™ is contraindicated in women who have:

1. A high FSH level indicating primary ovarian failure.
2. Uncontrolled thyroid and adrenal dysfunction.
3. An organic intracranial lesion such as pituitary tumor.
4. The presence of any cause of infertility other than anovulation.
5. Abnormal bleeding of undetermined origin.
6. Ovarian cysts or enlargement not due to polycystic ovary syndrome.
7. Prior hypersensitivity to urofollitropins, purified.
8. Bravelle™ is contraindicated in women who are pregnant and may cause fetal harm when administered to a pregnant woman. There are limited human data on the effects of Bravelle™ when administered during pregnancy.

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WARNINGS

Bravelle™ is a drug that should only be used by physicians who are thoroughly familiar with infertility problems. It is a potent gonadotropic substance capable of causing Ovarian Hyperstimulation Syndrome [OHSS] with or without pulmonary or vascular complications in women. Bravelle™ therapy requires a certain time commitment by physicians and supportive health professionals, and its use requires the availability of appropriate monitoring facilities (see PRECAUTIONS-Laboratory Tests). Bravelle™ should be used with a great deal of care.

Overstimulation of the Ovary During Bravelle™ Therapy

Ovarian Enlargement: Mild to moderate uncomplicated ovarian enlargement which may be accompanied by abdominal distension and/or abdominal pain occurs in approximately 20% of those treated with follitropin and hCG, and generally regresses without treatment within two or three weeks.

In order to minimize the hazard associated with the occasional abnormal ovarian enlargement, which may occur with FSH - hCG therapy, the lowest dose consistent with expectation of good results should be used. Careful monitoring of ovarian response can further minimize the risk of overstimulation.

If the ovaries are abnormally enlarged on the last day of Bravelle™ therapy, hCG should not be administered in the course of therapy; this will reduce the chances of development of the Ovarian Hyperstimulation Syndrome.

OHSS: OHSS is a medical event distinct from uncomplicated ovarian enlargement. OHSS may progress rapidly to become a serious medical event. It is characterized by an apparent dramatic increase in vascular permeability, which can result in a rapid accumulation of fluid in the peritoneal cavity, thorax, and potentially, the pericardium. The early warning signs of development of OHSS are severe pelvic pain, nausea, vomiting, and weight gain. The following symptomatology has been seen with cases of OHSS: abdominal pain, abdominal distension, gastrointestinal symptoms including nausea, vomiting and diarrhea, severe ovarian enlargement, weight gain, dyspnea, and oliguria. Clinical evaluation may reveal hypovolemia, hemoconcentration, electrolyte imbalances, ascites, hemoperitoneum, pleural effusions, hydrothorax, acute pulmonary distress, and thromboembolic events (see "Pulmonary and Vascular Complications" below). Transient liver function test abnormalities suggestive of hepatic dysfunction, which may be accompanied by morphologic changes on liver biopsy, have been reported in association with the Ovarian Hyperstimulation Syndrome (OHSS).

In a clinical study of ovulation induction, 6 of 72 (8.33%) Bravelle™ treated women developed OHSS and two were classified as severe. In a clinical study for multiple follicular development during IVF, 3 of 60 Bravelle™ treated women developed OHSS and 1 was classified as severe. Cases of OHSS are more common, more severe and more protracted if pregnancy occurs. OHSS develops rapidly; therefore patients should be followed for at least two weeks after hCG administration. Most often, OHSS occurs after treatment has been discontinued and reaches its maximum at about 7 to 10 days after treatment. Usually, in cases where OHSS may be developing prior to hCG administration (see PRECAUTIONS - Laboratory Tests), the hCG should be withheld.

If severe OHSS occurs, treatment **must** be stopped and the patient should be hospitalized.

A physician experienced in the management of the syndrome, or who is experienced in the management of fluid and electrolyte imbalances should be consulted.

Pulmonary and Vascular Complications

Serious pulmonary conditions (e.g. atelectasis, acute respiratory distress syndrome) have been reported. In addition, thromboembolic events both in association with, and separate from, the Ovarian Hyperstimulation Syndrome have been reported following FSH therapy. Intravascular thrombosis and embolism, which may originate in venous or arterial vessels, can result in reduced blood flow to critical organs or the extremities. Sequelae of such events have included venous thrombophlebitis, pulmonary embolism, pulmonary infarction, cerebral vascular occlusion (stroke), and arterial occlusion resulting in loss of limb. In rare cases, pulmonary complications and/or thromboembolic events have resulted in death.

Multiple Pregnancies

Multiple pregnancies have occurred following treatment with Bravelle™ SC and IM.

Pregnancy outcomes in a controlled study of 72 patients undergoing ovulation induction with Bravelle™ are shown in Table 4.

Table 4. FPI FSH 99-03 Outcome of Pregnancies

	Bravelle™ SC	Bravelle™ IM
Parameter	N(%)	N(%)
Total Continuing Pregnancies	9 (100)	7 (100)
Singlets	3 (33.3)	5 (71.4)
Total No. with Multiple Pregnancies	6 (66.7)	2 (28.6)
Twins	4	0
Triplets	2	0
Quadruplets	0	1
Quintuplets	0	0
Sextuplets	0	1

The pregnancy outcomes in a controlled study of 60 patients undergoing treatment with Bravelle™ in IVF are shown in Table 5.

Table 5. FPI FSH 2001-01 Outcome of Pregnancies

Bravelle™ SC	
Parameter	N(%)
Total No. of Continuing Pregnancies	23 (100)
Singlets	15 (65.2)
Total No. of Multiple Pregnancies	8 (34.8)
Twins	5
Triplets	3

The patient and her partner should be advised of the potential risk of multiple births before starting treatment.

Hypersensitivity/Anaphylactic Reactions

Hypersensitivity anaphylactic reactions associated with follitropins for injection, purified administration have been reported in some patients. These reactions presented as generalized urticaria, facial edema, angioneurotic edema, and/or dyspnea suggestive of laryngeal edema. The relationship of these symptoms to uncharacterized urinary proteins is uncertain.

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PRECAUTIONS

General

Careful attention should be given to the diagnosis of infertility in the selection of candidates for Bravelle™ therapy (see "**INDICATIONS AND USAGE**-Selection of patients").

Information for Patients

Prior to therapy with Bravelle™, patients should be informed of the duration of treatment and the monitoring of their condition that will be required. Possible adverse reactions (see ADVERSE REACTIONS section) and the risk of multiple births should also be discussed.

Laboratory Tests

The combination of both estradiol levels and ultrasonography are useful for monitoring the growth and development of follicles, timing hCG administration, as well as minimizing the risk of the Ovarian Hyperstimulation Syndrome and multiple gestations.

The clinical confirmation of ovulation, is determined by:

- A rise in basal body temperature,
- Increase in serum progesterone, and
- Menstruation following the shift in basal body temperature.

When used in conjunction with indices of progesterone production, sonographic visualization of the ovaries will assist in determining if ovulation has occurred. Sonographic evidence of ovulation may include the following:

- Fluid in the cul-de-sac,
- Ovarian stigmata, and
- Collapsed follicle.

Because of the subjectivity of the various tests for the determination of follicular maturation and ovulation, it cannot be overemphasized that the physician should choose tests with which he/she is thoroughly familiar.

Carcinogenesis Mutagenesis

Long-term toxicity studies in animals and in vitro mutagenicity tests have not been performed to evaluate the carcinogenic potential of urofollitropin for injection, purified.

Pregnancy

Pregnancy Category X: See **CONTRAINDICATIONS** section.

Nursing Mothers

It is not known whether this drug is excreted in human milk. Because many drugs are excreted in human milk and because of the potential for serious adverse reactions in the nursing infant from Bravelle™, a decision should be made whether to discontinue nursing or to discontinue the drug, taking into account the importance of the drug to the mother.

Pediatric Patients

Safety and effectiveness in pediatric patients have not been established.

Geriatric Patients

Safety and effectiveness in geriatric patients have not been established.

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ADVERSE REACTIONS

The safety of Bravelle™ was examined in four clinical studies that enrolled a total of 222 patients receiving Bravelle™ including 72 for ovulation induction and 150 for IVF.

All adverse events (without regard to causality assessment) occurring $\geq 2\%$ incidence in the clinical study patients receiving Bravelle™ are listed in Table 6, (FPI FSH 99-03 study for ovulation induction) and Table 7 (FPI FSH 99-04, FPI FSH 99-05 and FPI FSH 2001-01 studies for IVF).

Table 6. FPI FSH 99-03 Ovulation Induction Safety Profile

Patients with Adverse Events $\geq 2\%$		
Adverse Events (%)	Bravelle™ SC	Bravelle™ IM
	N = 35	N = 37
Genitourinary/Reproductive		
OHSS	4 (11.4)	2 (5.4)
Vaginal Hemorrhage	3 (8.6)	0 (0.0)
Ovarian Disorder (Pain, Cyst)	1 (2.9)	3 (8.1)
Urinary tract infection	0	1 (2.7)
Cervix disorder	1 (2.9)	0
Gastrointestinal		
Nausea	2 (5.7)	0 (0.0)
Enlarged Abdomen	1 (2.9)	1 (2.7)
Abdominal Pain	1 (2.9)	2 (5.4)
Vomiting	0	1 (2.7)

Constipation	0	1 (2.7)
Diarrhea	0	1 (2.7)
Metabolic/Nutritional		
Dehydration	0	1 (2.7)
Weight gain	1 (2.9)	0
Skin/Appendages		
Acne	1 (2.9)	0
Exfoliative dermatitis	0	1 (2.7)
Other Body Systems		
Headache	4 (11.4)	3 (8.1)
Pain	2 (5.7)	0 (0.0)
Neck Pain	0	1 (2.7)
Respiratory Disorder	2 (5.7)	0 (0.0)
Hot Flashes	2 (5.7)	0 (0.0)
Fever	0	1 (2.7)
Hypertension	0	1 (2.7)
Emotional lability	0	1 (2.7)
Depression	0	1 (2.7)
Accidental injury	0	1 (2.7)

Table 7. Integrated IVF Safety Profile

Patients with Adverse Events $\geq 2\%$	
Adverse Events (%)	Bravelle™ SC
	N=150
Genitourinary/Reproductive	
Vaginal hemorrhage	7 (4.7)
Post retrieval pain	12 (8.0)
Pelvic pain/cramps	10 (6.7)
OHSS	9 (6.0)
Uterine spasms	4 (2.7)
Vaginal spotting	4 (2.7)
Urinary tract infection	5 (3.3)
Ovarian disorder	3 (2.0)
Breast tenderness	3 (2.0)

Vaginal Discharge	4 (2.7)
Infection fungal	3 (2.0)
Gastrointestinal	
Abdominal cramps	21 (14.0)
Nausea	13 (8.7)
Abdominal pain	7 (4.7)
Abdominal fullness/enlargement	10 (6.7)
Constipation	3 (2.0)
Other Body Systems	
Headache	19 (12.7)
Pain	8 (5.3)
Rash	4 (2.7)
Respiratory disorder	6 (4.0)
Sinusitis	3 (2.0)
Injection site reaction	6 (4.0)
Hot flash	6 (4.0)
Emotional lability	3 (2.0)

The following medical events have been reported subsequent to pregnancies resulting from gonadotropin therapy in published clinical studies:

1. Spontaneous Abortion
2. Ectopic Pregnancy
3. Premature Labor
4. Postpartum fever
5. Congenital abnormalities

The following adverse reactions have been previously reported during urofollitropin for injection, purified therapy:

1. Pulmonary and vascular complications (see **WARNINGS**)
2. Adnexal torsion (as a complication of ovarian enlargement),
3. Mild to moderate ovarian enlargement,
4. Hemoperitoneum
5. There have been infrequent reports of ovarian neoplasms, both benign and malignant, in women who have undergone multiple drug regimens for ovulation induction; however, a causal relationship has not been established.

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DRUG ABUSE AND DEPENDENCE

There have been no reports of abuse or dependence with follitropins.

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OVERDOSAGE

Aside from possible ovarian hyperstimulation (see WARNINGS) and multiple gestations (see WARNINGS), little is known concerning the consequences of acute overdosage with Bravelle™.

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DOSAGE AND ADMINISTRATION

Dosage:

Infertile patients with oligo-anovulation:

The dose of Bravelle™ to stimulate development of ovarian follicles must be individualized for each patient. The lowest dose consistent with achieving good results based on clinical experience and reported clinical data should be used.

The recommended initial dose of Bravelle™ for patients who have received GnRH agonist or antagonist suppression is 150 IU daily administered SC or IM for the first 5 days of treatment. Based on clinical monitoring (including serum estradiol levels and vaginal ultrasound results) subsequent dosing should be adjusted according to individual patient response. Adjustments in dose should not be made more frequently than once every 2 days and should not exceed more than 75 to 150 IU per adjustment. The maximum daily dose of Bravelle™ should not exceed 450 IU and in most cases dosing beyond 12 days is not recommended.

If patient response to Bravelle™ is appropriate, hCG (5000 to 10,000 USP units) should be given 1 day following the last dose of Bravelle™. The hCG should be withheld if the serum estradiol is greater than 2000 pg/mL, if the ovaries are abnormally enlarged or if abdominal pain occurs, and the patient should be advised to refrain from intercourse. These precautions may reduce the risk of Ovarian Hyperstimulation Syndrome and multiple gestations. Patients should be followed closely for at least 2 weeks after hCG administration. If there is inadequate follicle development or ovulation without subsequent pregnancy, the course of treatment with Bravelle™ may be repeated. The couple should be encouraged to have intercourse daily, beginning on the day prior to the administration of hCG until ovulation becomes apparent from the indices employed for the determination of progestational activity. In the light of the foregoing indices and parameters mentioned, it should become obvious that, unless a physician is willing to devote considerable time to these patients and be familiar with and conduct the necessary laboratory studies, he/she should not use Bravelle™.

Assisted Reproductive Technologies:

The recommended initial dose of Bravelle™ for patients undergoing IVF and donor egg patients who have received GnRH agonist or antagonist pituitary suppression is 225 IU daily administered SC for the first 5 days of treatment. Based on clinical monitoring (including serum estradiol levels and vaginal ultrasound results) subsequent dosing should be adjusted according to individual patient response. Adjustments in dose should not be made more frequently than once every 2 days and should not exceed more than 75 to 150 IU per adjustment. The maximum daily dose of Bravelle™ given should not exceed 450 IU and in most cases dosing beyond 12 days is not recommended.

Once adequate follicular development is evident, hCG (5000-10,000 USP units) should be administered to induce final follicular maturation in preparation for oocyte retrieval. The administration of hCG must be withheld in cases where the ovaries are abnormally enlarged on the last day of therapy. This should reduce the chance of developing OHSS.

Directions for Using Bravelle™

1. Wash hands thoroughly with soap and water.
2. Before injections, the septum tops of the vials should be wiped with an aseptic solution to prevent contamination of the contents.
3. To prepare the Bravelle™ solution, inject 1 mL of Sterile Saline for Injection, USP into the vial of Bravelle™. **DO NOT SHAKE**, but gently swirl until the solution is clear. Generally, the Bravelle™ dissolves immediately. Check the liquid in the container. If it is not clear or has particles in it, **DO NOT USE IT**.
4. For patients requiring a single injection from multiple vials of Bravelle™, up to 6 vials can be reconstituted with 1 mL of Sterile Saline for Injection, USP. This can be accomplished by reconstituting a single vial as described above (see step 3). Then draw the entire contents of the first vial into a

syringe, and inject the contents into a second vial of lyophilized Bravelle™. Gently swirl the second vial, as described above, once again checking to make sure the solution is clear and free of particles. This step can be repeated with 4 additional vials for a total of up to 6 vials of lyophilized Bravelle™ into 1 mL of diluent.

5. Immediately **ADMINISTER** the reconstituted Bravelle™ either **SC** (for ovulation induction or multifollicular development during ART) or **IM** (for ovulation induction). Any unused reconstituted material should be discarded.
6. Draw the reconstituted Bravelle™ Into an empty, sterile syringe.
7. Hold the syringe pointing upwards and gently tap the side to force any air bubbles to the top; then squeeze the plunger gently until all the air has been expelled and only Bravelle™ solution is left in the syringe.
8. Bravelle™ works if it is injected **SC** (for ovulation induction or multifollicular development during ART) or **IM** (for ovulation induction). The recommended sites for SC injection are either side of the lower abdomen in alternating fashion with the actual injection site varied a little with each injection. SC injection of Bravelle™ into the thigh is not recommended unless the lower abdomen is not usable because of scarring, surgical deformity or other medical conditions.

The best site for intramuscular injection of Bravelle™ is the upper outer quadrant of the buttock muscle near the hip. This area contains few blood vessels and major nerves. Stretching the skin helps the needle to go in more easily and pushes the tissue beneath the skin out of the way. This helps the solution disperse correctly.

9. The injection site should be swabbed with a disinfectant to remove any surface bacteria. Clean about two inches around the point where the needle will go in and let the disinfectant dry for at least one minute before proceeding.
10. For **SC** injection, the needle should be inserted at a 90° angle to the skin surface. For **IM** injection, the needle should be inserted at a 90° angle to the skin surface. Pushing in with a quick thrust causes the least discomfort.
11. If the needle is correctly positioned, it will be difficult to draw back on the plunger. Any blood drawn into the syringe means the needle tip has penetrated a vein or artery. If this happens, remove the syringe, cover the injection site with a swab containing disinfectant and apply pressure; the site should stop bleeding in a minute or two.
12. Once the needle is properly placed, depress the plunger **slowly** and steadily, so the solution is correctly injected and the skin or muscle tissue is not damaged.
13. Pull the syringe out quickly and apply pressure to the site with a swab containing disinfectant. A gentle massage of the site - while still maintaining pressure - helps disperse the Bravelle™ solution and relieve any discomfort.
14. Use the disposable syringe only once and dispose of it properly.

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HOW SUPPLIED

Bravelle™ (urofollitropin for injection, purified) is supplied in a sterile, lyophilized, single dose vial containing 82.5 IU of FSH, to deliver 75 IU FSH after reconstituting with the diluent.

Each vial is available with an accompanying vial of sterile diluent containing 2 mL of 0.9% Sodium Chloride Injection, USP.

75 IU FSH activity, supplied as:

NDC 55566-8505-2: Box of 5 vials + 5 vials diluent.

NDC 55566-8505-3: Box of 100 vials + 100 vials diluent

Lyophilized powder may be stored refrigerated or at room temperature (3° to 25° C/37° to 77°F). Protect from light. Use immediately after reconstitution. Discard unused material.

Rx only

Vials of sterile diluent of 0.9% Sodium Chloride Injection, USP, manufactured for Ferring Pharmaceuticals Inc.

Manufactured for:
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SUFFERN, NY 10901
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FDA APPROVES BRAVELLE™
(urofollitropin for injection, purified)

**FERRING'S NEW HUMAN-DERIVED FOLLICLE-STIMULATING
HORMONE FOR INFERTILITY TREATMENT**

**New Highly Purified Alternative To Genetically Engineered Infertility
Treatments**

TARRYTOWN, NY – May 6, 2002 – Ferring Pharmaceuticals, a world leader in naturally occurring protein hormones, announced today that it has received approval from the U.S. Food and Drug Administration (FDA) to market Bravelle™ (urofollitropin for injection, purified), a highly purified, human-derived follicle-stimulating hormone (hFSH) for the treatment of infertility. Bravelle™, in conjunction with human chorionic gonadotropin, is indicated for ovulation induction following pituitary suppression.

"With the introduction of Bravelle™, Ferring has expanded its family of human-derived hormones to include a highly purified, well-tolerated hFSH with proven efficacy in ovulation induction, a critical step in many infertility treatment protocols," said Wayne Anderson, president of Ferring Pharmaceuticals. "Based on the fact recombinant technology has shown no meaningful advantage in either efficacy or safety in the clinic, Ferring remains committed to the development of human-derived products in order to seek improvements in ovarian stimulation protocols. Ferring has submitted an application to the FDA seeking additional indications for Bravelle™ in infertility treatment. This application, which is supported by additional clinical studies, brings the total number of patients studied to 577. This application is currently under review by the FDA."

**A Human-Derived FSH Proven as Safe and Effective as Genetically
Engineered FSH**

FPI007877

Bravelle™ was compared to follitropin beta, a recombinant FSH, in a prospective, parallel group, multicenter trial in 111 oligo-anovulatory patients undergoing ovulation induction. Patients underwent pituitary suppression with a GnRH agonist prior to being randomized to Bravelle™ SC, Bravelle™ IM or follitropin beta SC. Results showed that there were no significant differences in efficacy and safety between the treatment groups.

Percentage of patients achieving:	Bravelle™ SC (n=26)	Follitropin beta SC (n=35)
Ovulation	96.1%	85.7%
Clinical pregnancy	34.6%	31.4%
Continuing pregnancy	34.6%	28.6%
Live birth	34.6%	17.1%

In addition to the studies supporting the new drug application, Ferring has recently completed two Phase 3B clinical trials involving 24 centers. These trials evaluated the use of Bravelle™ together with Repronex® (mixed protocol), Ferring's human menopausal gonadotropin in the same syringe, in two age groups. The first study evaluated the use of a mixed protocol in 108 women ages 18 to 33 years; the second trial evaluated 120 women ages 34 to 40 years. This is the first time a prospective, systematic clinical evaluation of single daily dose mixed protocols has been conducted anywhere in the world.

Bravelle™: The Natural Choice

Bravelle™ is affordably priced, an important benefit since infertility treatment is generally not fully covered by insurance. It is available for both subcutaneous and intramuscular injection. Most patients prefer SC administration because it is more convenient and causes less discomfort. Added Anderson, "Bravelle™ is ideally suited to

FPI007878

meet the needs of infertility specialists and their patients by providing an affordable solution that combines human-derived hormone efficacy with recombinant hormone-like purity."

Only physicians thoroughly familiar with Infertility treatment, including the risk of multiple births and adverse reactions, should prescribe Bravelle™. Like all gonadotropins, Bravelle™ is a potent substance capable of causing mild to severe adverse reactions, including ovarian hyperstimulation syndrome (incidence of 8.2%), with or without pulmonary vascular complications, in women undergoing therapy for infertility.

Background on Human-Derived Hormones

The key differences in human-derived and genetically engineered infertility treatments are raw material sources and cost. Human-derived FSH treatments are highly purified follitropins extracted from the urine of postmenopausal women. By comparison, genetically engineered products are derived from the secretions from Chinese hamster ovary cells that are cultured in fetal calf or other mammalian serum, and approximate human hormones. Both are manufactured in compliance with extremely strict standards (including viral inactivation and confirmatory testing), but human-derived products are generally less expensive than their genetically engineered counterparts.

About Ferring

Ferring Pharmaceuticals, part of the Ferring Group, a privately owned, international pharmaceutical company, markets Bravelle™, Repronex® and Novarel® in the U.S. to infertility specialists and their patients. The Ferring Group specializes in the research, development and commercialization of compounds in general and pediatric endocrinology, urology, gastroenterology, obstetrics/gynecology and infertility.

For more information, call 1-888-337-7484 or visit www.ferringusa.com.

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For full prescribing information contact Kelly Laban at:

203-762-8833.

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Ferring Pharmaceuticals Presents Data on New Infertility Treatments at the Pacific Coast Reproductive Society Meeting

4/22/2002 8:03:00 AM

RANCHO LAS PALMAS, Calif., Apr 22, 2002 /PRNewswire via COMTEX/ — Results of clinical trials evaluating Ferring Pharmaceuticals' infertility treatments — a human-derived follicle stimulating hormone (hFSH) and a purified human menopausal gonadotropin (hMG), which are currently under investigation — were presented at the 50th Annual Pacific Coast Reproductive Society Meeting in Rancho Las Palmas, CA, April 17 to 21, 2002. Four clinical abstracts and two posters were presented.

"These new clinical studies reflect Ferring's commitment to broadening the existing body of knowledge in the field of infertility, while also providing meaningful data that reflect current treatment trends," said Wayne Anderson, president of Ferring Pharmaceuticals Inc. "As specialists in the development of human-derived hormone medications, we look forward to offering physicians even greater treatment flexibility when we receive approval of our new hFSH — the fourth addition to our family of human-derived hormones."

Same Syringe Mixed Protocol with Diluent

Ferring's hFSH and purified hMG were tested to determine if the bioactivities of FSH and LH contained in the two treatments are altered after reconstituting them in the same diluent and mixing them in the same plastic syringe.

"This is the first time that FSH and hMG mixed in the same syringe has been tested using sophisticated bioassays," said Mahendra DeSilva, Ph.D., Ferring's manager of Professional Services. "Mixed protocols for ovarian stimulation are widely-used, and often combine FSH and hMG in the same syringe to decrease the number of daily injections. Once approved, infertility specialists will be able to mix Ferring's hFSH and purified hMG in the same syringe with the assurance that the bioactivity of FSH and LH remains unchanged."

Separate low, medium and high dose bioassays were performed in duplicate, comparing Ferring's hFSH, purified hMG and FSH and LH Reference Standards. The study demonstrated that Ferring's hFSH and purified hMG can be reconstituted in 0.9% sodium chloride and combined in the same syringe with no alterations in the bioactivity of FSH or LH.

Ferring's Purified hMG Comparative Study

Ferring's purified hMG was evaluated in a prospective, randomized, parallel group, open-label,

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FPI007918

multicenter study that compared purified hMG administered subcutaneously (SC) and intramuscularly (IM) to Repronex administered SC in patients undergoing in vitro fertilization. The primary measure of efficacy was the number of oocytes retrieved. Results were positive and were described in detail in posters presented at the meeting.

About Repronex and Ferring

Repronex is the only human menopausal gonadotropin (hMG) approved for both subcutaneous and intramuscular administration. It is also the most frequently prescribed hMG. Repronex, like all gonadotropins, is a potent substance capable of causing mild to severe adverse reactions, including OHSS (incidence of 3.5%), with or without pulmonary or vascular complications, in women undergoing therapy for infertility. Only physicians thoroughly familiar with infertility treatment, including the risk of multiple births and adverse reactions, should prescribe Repronex.

Ferring Pharmaceuticals Inc., headquartered in Tarrytown, NY, is part of the Ferring Group, a privately owned, international biopharmaceutical company that specializes in the research, development and commercialization of compounds in general and pediatric endocrinology, urology, gastroenterology, obstetrics/gynecology and infertility. For more information, call 1-888-337-7464 or visit the Ferring Web site at <http://www.ferringusa.com>.

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News from PR Newswire

Ferring Pharmaceuticals Presents Data on New Infertility Treatments at the Pacific Coast Reproductive Society Meeting

PR Newswire - Monday April 22, 2002

RANCHO LAS PALMAS, Calif., April 22 /PRNewswire/ -- Results of clinical trials evaluating Ferring Pharmaceuticals' infertility treatments -- a human-derived follicle stimulating hormone (hFSH) and a purified human menopausal gonadotropin (hMG), which are currently under investigation -- were presented at the 50th Annual Pacific Coast Reproductive Society Meeting in Rancho Las Palmas, CA, April 17 to 21, 2002. Four clinical abstracts and two posters were presented.

"These new clinical studies reflect Ferring's commitment to broadening the existing body of knowledge in the field of infertility, while also providing meaningful data that reflect current treatment trends," said Wayne Anderson, president of Ferring Pharmaceuticals Inc. "As specialists in the development of human-derived hormone medications, we look forward to offering physicians even greater treatment flexibility when we receive approval of our new hFSH -- the fourth addition to our family of human-derived hormones."

Same Syringe Mixed Protocol with Diluent

Ferring's hFSH and purified hMG were tested to determine if the bioactivities of FSH and LH contained in the two treatments are altered after reconstituting them in the same diluent and mixing them in the same plastic syringe.

"This is the first time that FSH and hMG mixed in the same syringe has been tested using sophisticated bioassays," said Mahendra DeSilva, Ph.D., Ferring's manager of Professional Services. "Mixed protocols for ovarian stimulation are widely-used, and often combine FSH and hMG in the same syringe to decrease the number of daily injections. Once approved, infertility specialists will be able to mix Ferring's hFSH and purified hMG in the same syringe with the assurance that the bioactivity of FSH and LH remains unchanged."

Separate low, medium and high dose bioassays were performed in duplicate, comparing Ferring's hFSH, purified hMG and FSH and LH Reference Standards. The study demonstrated that Ferring's hFSH and purified hMG can be reconstituted in 0.9% sodium chloride and combined in the same syringe with no alterations in the bioactivity of FSH or LH.

Ferring's Purified hMG Comparative Study

FPI007920

Ferring's purified hMG was evaluated in a prospective, randomized, parallel group, open-label, multicenter study that compared purified hMG administered subcutaneously (SC) and intramuscularly (IM) to Repronex administered SC in patients undergoing in vitro fertilization. The primary measure of efficacy was the number of oocytes retrieved. Results were positive and were described in detail in posters presented at the meeting.

About Repronex and Ferring

Repronex is the only human menopausal gonadotropin (hMG) approved for both subcutaneous and intramuscular administration. It is also the most frequently prescribed hMG. Repronex, like all gonadotropins, is a potent substance capable of causing mild to severe adverse reactions, including OHSS (incidence of 3.5%), with or without pulmonary or vascular complications, in women undergoing therapy for infertility. Only physicians thoroughly familiar with infertility treatment, including the risk of multiple births and adverse reactions, should prescribe Repronex.

Ferring Pharmaceuticals Inc., headquartered in Tarrytown, NY, is part of the Ferring Group, a privately owned, international biopharmaceutical company that specializes in the research, development and commercialization of compounds in general and pediatric endocrinology, urology, gastroenterology, obstetrics/gynecology and infertility. For more information, call 1-888-337-7464 or visit the Ferring Web site at <http://www.ferringusa.com>.

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SOURCE Ferring Pharmaceuticals Inc.

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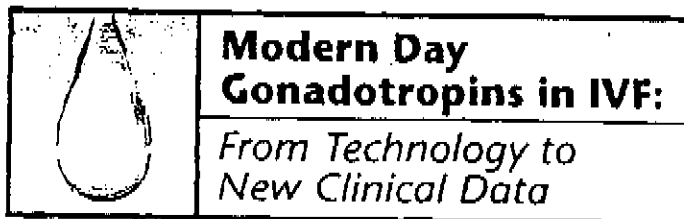
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FP1007921

*Continuing medical education
breakfast symposium sponsored by the
American Society for Reproductive Medicine
during the ASRM 57th Annual Meeting:*



The Peabody Orlando
Orlando, Florida
Monday, October 22, 2001

CO-CHAIRS:

William Keye, Jr., MD
Zev Rosenwaks, MD

FACULTY:

Delphine Lévy, MD
Richard Marrs, MD
Richard Scott, MD
Michael Steinkampf, MD



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FERRING
PHARMACEUTICALS

FP1027485

Modern Day Gonadotropins In IVF

From Technology to New Clinical Data

ACKNOWLEDGEMENTS

This symposium is being supported through an unrestricted educational grant from Ferring Pharmaceuticals.

DISCLOSURE

Each speaker is required to disclose the existence of any financial interest and/or other relationships that may have with the manufacturers of any commercial products to be addressed during his/her presentation and/or the commercial contributions of this activity.

Dr. William Keye, Jr.

Receives grants and contracts from Ferring Pharmaceuticals and Serono Laboratories. Receives honoraria or consultation fees from Ferring Pharmaceuticals, Pfizer Inc. and Eli Lilly and Company. Member of a company advisory board, board of directors or other similar group for Organon and Ferring Pharmaceuticals.

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Receives grants and contracts from Serono Laboratories and Organon. Receives honoraria or consultation fees from Serono Laboratories.

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Dr. Delphine Lévy

None

Dr. Richard Marris
Receives honoraria or consultation fees from Ferring Pharmaceuticals and Serono Laboratories.

Dr. Richard Scott

Participates in the following company-sponsored speaker's bureau: Ferring Pharmaceuticals, Serono Pharmaceuticals and Organon.

Dr. Michael Steinkampf

Receives grants and contracts from Ferring Pharmaceuticals and Serono Laboratories.

Dr. Richard Marris

Receives honoraria or consultation fees from Ferring Pharmaceuticals and Serono Laboratories.

None of the faculty will be discussing any off-label or otherwise non-approved uses of any product.

Modern Day Gonadotropins In IVF

From Technology to New Clinical Data

PROGRAM AGENDA

6:00-6:15 AM

Opening Remarks, Disclosure, Current Issues in Gn In IVF Dr. William Keye, Jr.
Director, Division of Reproductive Endocrinology and Infertility
Robert H. Lurie Medical Research Center
Department of Obstetrics and Gynecology
University of Michigan
Ann Arbor, Michigan

Introduction of Faculty

6:15-6:35 AM

Human-Derived Technology vs. Recombinant Technology

..... Dr. Zey Rosenwaks
Ferring Pharmaceuticals, Gynecology and Reproductive Medicine
Weill Medical College of Cornell University
New York, New York

6:35-6:55 AM

The Role of LH in Follicular Growth and Ovulation

..... Dr. Delphine Lévy
Reproductive Medicine Division of New Jersey
Morristown, New Jersey

6:55-7:15 AM

Results of FSH IVF Clinical Trials

..... Dr. Michael Steinkampf
Reproductive Medicine Division of New Jersey
Morristown, New Jersey

7:15-7:35 AM

Stability Data and Mixed Protocol Results

..... Dr. Richard Marris
Medical Director
Center for Assisted Reproductive Medicine
at Serono Laboratories, Inc.
Kenilworth, New Jersey

7:35-7:45 AM

Questions and Answers

..... Dr. Zey Rosenwaks

FPI027486

Zev Rosenwaks, MD

PROFILE

Zev Rosenwaks, MD is the Director of The Center for Reproductive Medicine and Infertility, the world-renowned infertility clinic at New York Weill Cornell. He is Professor of Obstetrics and Gynecology at Weill Medical College of Cornell University and was appointed an endowed professorship in 1994—the Rankin Distinguished Professor of Reproductive Medicine in Obstetrics and Gynecology. Dr. Rosenwaks is a diplomate of the American Board of Obstetrics and Gynecology and received his subspecialty certification in Reproductive Endocrinology in 1981. He is a noted world authority on reproductive endocrinology and infertility and one of the founding pioneers in the assisted reproductive technologies.

Throughout his career, Dr. Rosenwaks has been instrumental in developing new fertility-enhancing protocols and the study of advanced recombinant-derived gonadotropins to assist in producing mature, high quality sperm and eggs. The development of a highly successful egg donation program at New York Weill Cornell has made it possible to achieve pregnancies in women who have premature ovarian failure. Egg donation, first developed in the United States by Dr. Rosenwaks, has also made it possible to answer many key questions about human reproduction.

At New York Weill Cornell's The Center for Reproductive Medicine and Infertility, patients may avail themselves of a myriad of reproductive options ranging from in vitro fertilization (IVF), micropipette sperm aspiration (MCS), preimplantation genetic testing, ovulation induction and premarin immunization (PMI), to participation in use of

the most successful egg donation programs in the country. The center provides a full spectrum of state-of-the-art medical and surgical treatment in reproductive endocrinology and gynecology including laser surgery, tubal reconstruction, laparoscopy and robotic gynecology, as well as treatment of disorders of sexual development and in-house psychological counseling. Close collaboration with New York Weill Cornell's Weill Center for Reproductive Medicine and Microsurgery makes Dr. Rosenwaks' program unique for its treatment of male and female reproductive problems.

Dr. Rosenwaks and his world-class team of reproductive endocrinologists, embryologists, anesthesiologists and infertility specialists have helped more couples have babies through assisted reproduction than any other center in the country, and they have consistently achieved among the highest success rates in the world. Dr. Rosenwaks has had quite a prolific career—he has authored over 200 scientific papers, 42 book chapters and 5 textbooks, the latest of which is a comprehensive two-volume text, entitled, "Reproductive Endocrinology, Surgery, and Technology" available from Lippincott-Raven Publishers.

William R. Keye, Jr., MD

PROFILE

William Keye, Jr., MD, is certified by the American Board of Obstetrics and Gynecology and by his subspecialty division of Reproductive Endocrinology.

A graduate of the University of Minnesota Medical School, Dr. Keye completed his residency training in obstetrics and gynecology at both the University of Minnesota and the University of California—San Francisco (UCSF). Dr. Keye completed a reproductive endocrinology research program at the University of Michigan, followed by a fellowship in reproductive endocrinology at UCSF. He is Board Certified in Obstetrics and Gynecology, Reproductive Endocrinology and Infertility.

After two years in the U.S. Air Force Medical Corps, Dr. Keye spent two years in private obstetrics and gynecology practice in Indiana. Dr. Keye was a member of the University of Utah Medical School faculty from 1979 to 1990, when he joined William Beaumont Hospital in Royal Oak as the director of the Division of Reproductive Endocrinology and In Vitro Fertilization in the Department of Obstetrics and Gynecology.

Dr. Keye is an active member of the American College of Obstetricians and Gynecologists and the American Society for Reproductive Medicine. He is a charter member of the Society of Reproductive Endocrinologists and the Society of Reproductive Surgeons. Dr. Keye was elected president of the Society of Reproductive Surgeons and the Michigan Society of Reproductive Endocrinologists for 1994 and is on the Board of Directors of the American Society for Reproductive Medicine and Society of Reproductive Surgeons. He will be President in 2002.

Dr. Keye has special expertise in female infertility and gynecologic reproductive endocrinology, including infertility, ovulation induction, IVF, reproductive surgery, hysterectomies, repetitive pregnancy loss, abnormal uterine anatomy, recurrent miscarriages, premenstrual syndrome, excessive hair growth, abnormal lactation, and menopause. He has authored or edited textbooks on the subjects of infertility, premenstrual syndrome, and laparoscopic laser surgery for gynecologic disorders and has written more than 300 scientific articles.

Dr. Keye is active in the community through his involvement in fund raising for women's health and is a member of the Beaumont Speakers Bureau. He speaks more than 50 times a year to professional and lay groups. As the result of these activities and his medical expertise, he has been cited in four Doctors in America, Town and Country, Good Housekeeping, Detroit Monthly and the Detroit Free Press.

He is on staff at William Beaumont Hospital in Royal Oak.

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William B. Keys, Jr., MD

ABSTRACT

CURRENT ISSUES IN OVULATION INDUCTION IN IVF

Prior to 1980 gonadotropins were used by a small number of specialists in a small number of infertile patients who wished to conceive. However, with the emergence of IVF as a popular treatment of infertility the use of gonadotropins increased dramatically.

With increasing experience with gonadotropins for IVF and improved methods for the preparation of sperm for insemination, physicians caring for infertile couples began to use gonadotropins for controlled ovarian hyperstimulation, prior to mixed intercourse or intravaginal insemination. As a result, gonadotropins use has increased dramatically during much of the last 10-15 years.

For most of the past 30 years we have used urinary derived human menopausal gonadotropins, which have been a mixture of LH and FSH. However, in the late 1980's products were introduced with less LH and fewer non-specific proteins. The introduction of a variety of gonadotropin preparations occurred for a number of reasons. Some were related to clinical concerns while others were proprietary.

The introduction of a variety of gonadotropins also has stimulated thoughtful clinicians and researchers to re-examine the role of gonadotropins in follicular stimulation and to search for the optimal gonadotropin preparation and the optimal protocol.

This seminar will address many of the issues and concerns that we as clinicians face every day in our practices. As co-chair of this symposium, Zeynep Adiguzel and I have put together a blue-ribbon panel of experts in ovulation and assisted reproductive technology to address these issues.

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Issues such as:
1. What are the relative roles of LH and FSH in follicular growth and ovulation? How much LH is necessary? What are the clinical implications of products with and without LH? Is too much LH harmful?

2. What are the differences and similarities between recombinant Chinese hamster ovary derived and urinary-based human derived gonadotropins?

3. What are the differences in clinical effectiveness of add bolus, basic bolus or an unrestricted combination of bolus?

4. What are the relative purities and safety profiles of recombinant and urinary-derived FSH preparations?

5. Are the questions about a limited supply of human-derived gonadotropins and batch-to-batch variability pertinent and important?

6. Are there significant differences in pregnancy rates following the use of either human-derived gonadotropins or recombinant FSH?

7. Finally, is there an ideal ratio of FSH:LH that is superior to others?

I think we'll find this symposium stimulating, thought-provoking and provocative. Hopefully it will challenge some of our beliefs about the use of gonadotropins and lead to a more rational evidence-based approach to ovulation induction and controlled ovarian stimulation.

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Richard T. Scott, Jr., MD, FACOG, HCLD

PROFILE

Dr. Scott, a board certified reproductive endocrinologist, is one of only a handful of physicians who are board certified by the American Board of Obstetrics and Gynecology (ABOG), endocrinology, and high complexity clinical laboratory director. The dual certification makes him uniquely suited to integrate both the clinical and laboratory services in a reproductive medicine practice. Dr. Scott and his partner established Reproductive Medicine Associates (RMA) in 1999. RMA is a private practice infertility and IVF program located in Teaneck, New Jersey. He previously was Director of the Advanced Reproductive Technology Program at the Institute for Reproductive Medicine and Science of Saint Barnabas Medical Center, Livingston, New Jersey. He completed his fellowship in reproductive endocrinology at the Johns Hopkins in Norfolk, Virginia, after receiving his M.D. degree from the University of Virginia Medical School. He was Clinical Director of the Reproductive Endocrinology Fellowship Program at the National Institutes of Health, Bethesda, Maryland, and previously founded the first IVF program in the federal government at Wilford Hall Medical School in San Antonio, Texas. At this center, he was personally responsible for all aspects of the program, serving as

physician, embryologist, and endocrinologist. Dr. Scott has completed more than 7,000 in vitro fertilization (IVF) cycles. He is an award-winning researcher and author of more than 200 scientific papers and abstracts, serves as an ad hoc reviewer for more than a dozen peer-reviewed journals, and has lectured on his research to reproductive endocrinologists throughout the United States and abroad. He has received more than a dozen awards for excellence in clinical and laboratory research, and is the recipient of the 1997 Chapter Award of ISOGVE of New York City and the American College of Obstetrics and Gynecology Professor of the Year Award. Dr. Scott is a recognized leader in the areas of ovulation induction for assisted reproduction, implantation, and ovarian reserve screening and oocyte donation. A lecturer for on national television broadcasts, he was named to Good Housekeeping's Best Doctor List in 1998.

Richard T. Scott, Jr., MD, FACOG, HCLD

ABSTRACT

HUMAN-DERIVED TECHNOLOGY VS. RECOMBINANT TECHNOLOGY

Recent improvements in the production of gonadotropins now provide clinicians with a variety of treatment options for their patients undergoing controlled ovarian hyperstimulation or in vitro fertilization. These advances have provided solutions to some of the traditional problems associated with the exogenous gonadotropins. The central issues when selecting a gonadotropin preparation for stimulation include consistency, purity, safety, and clinical efficacy.

Contemporary techniques used in producing human-derived gonadotropins have significantly reduced the "batch-to-batch" variability in gonadotropin potency. Purification techniques have further advanced the process and now allow the production of gonadotropins that are over 95% pure. The highly-purified, human-derived gonadotropins are similar in purity to the recombinant gonadotropins.

The safety of both human-derived and recombinant gonadotropins now appears established. Human-derived products do not require exposure to animal serum products. The production process of both recombinant products require exposure to animal serum products. Virtually all mammalian cell lines perform best in the presence of serum and bovine serum used in the culture system used to produce the recombinant gonadotropins. Both human-derived and recombinant gonadotropins go through a rigorous purification process designed to extract bacterial and viral particles. The process is highly effective and high standards of quality control are maintained and verified.


The clinical effectiveness of these medications is now well established. Widespread utilization in a large number of centers has resulted in excellent clinical responses and outcomes. Studies comparing outcomes to determine the single best medication or production process have produced mixed results. Clinicians should reflect upon their own experiences with the specific treatment protocols, which have been most effective in their own centers. Differences in clinical outcomes, if any, are likely to be quite small. Clinicians should also evaluate the overall cost effectiveness of each product when making treatment decisions.

FPI027489

Modern Day Gonadotropins in IVF

From Technology to New Clinical Data

HUMAN-DERIVED VS. RECOMBINANT TECHNOLOGY




Richard T. Scott, Jr., MD
Reproductive Medicine Associates of New Jersey
Basking Ridge, NJ 07004

Urinary versus Recombinant hCG
How do we decide?????


- Convenience
- Flexibility
- Reproductive Risk
- Gonadotropin source
- Chemical structure
- Chemical composition
- Immunogenicity
- Stability

Human Derived Technologies
The Original Standard




- Intermediate 75 IU
- Amplification
- Stability
- Highly variable
- Unpredictable
- Unreliable
- Unpredictable
- Unreliable

REPROX'S DOCUMENTED BATCH-TO-BATCH CONSISTENCY



Contemporary Production Standards for hMG have Increased Consistency in Dosing



HUMAN DERIVED VS. RECOMBINANT GONADOTROPINS

What are the differences?

- Structure
- Manufacturing
- Physiological response
- Chemical differences
- Outcome

Modern Day Gonadotropins in IVF

From Technology to New Clinical Data

HUMAN DERIVED VS. RECOMBINANT GONADOTROPINS - ISOFORMS

- Gonadotropins are large glycoproteins
- There is variability in the content of the glycoproteins
- Different isoforms are typically distinguished by the number of glycosylation sites and the number of amino acids in the peptide chain
- hCG is not a single molecule. It is a heterodimeric protein consisting of two subunits, alpha and beta.

HUMAN DERIVED VS. RECOMBINANT GONADOTROPINS COMPARING THE TECHNOLOGIES

Parameter	Human Derived	Recombinant
Structure	Identical	Identical
Function	Identical	Identical
Stability	Identical	Identical
Immunogenicity	Identical	Identical
Consistency	Identical	Identical


HUMAN DERIVED VS. RECOMBINANT GONADOTROPINS COMPARING THE TECHNOLOGIES

- Manufacturing process
- Consistency
- Stability
- Immunogenicity
- Consistency

HUMAN DERIVED VS. RECOMBINANT GONADOTROPINS COMPARING THE TECHNOLOGIES

- hCG preparations are produced by various methods and have different characteristics
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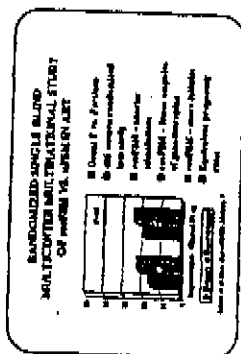
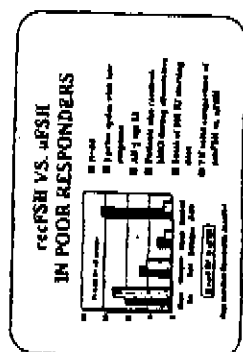
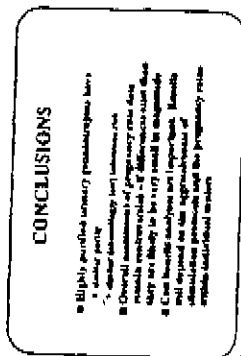
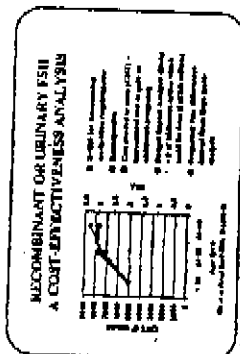
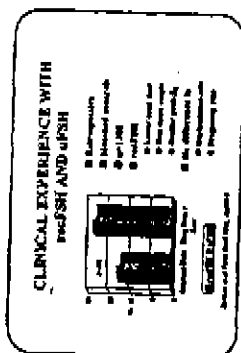
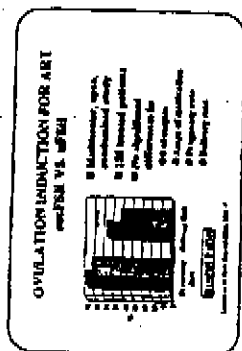
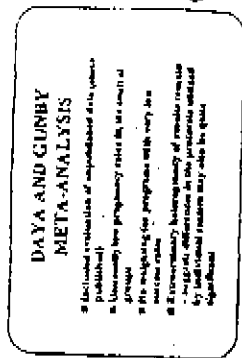
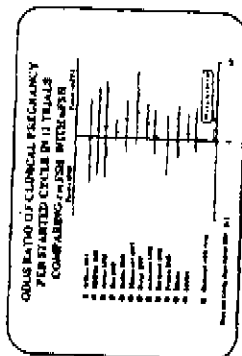
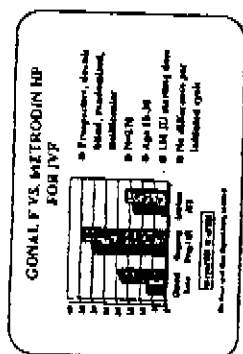
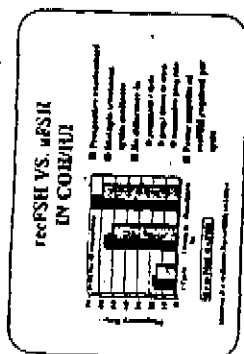
HUMAN DERIVED AND RECOMBINANT GONADOTROPINS COMPARING THE TECHNOLOGIES



DIFFERENCES BETWEEN JENSEN AND FISHER'S IN THE TYPE OF ISOFORMS AND THE SPECTRUM OF THE ISOFORMS

Parameter	Jensen	Fisher
Structure	Identical	Identical
Function	Identical	Identical
Stability	Identical	Identical
Immunogenicity	Identical	Identical
Consistency	Identical	Identical

FPI027490



Richard T. Scott, Jr., MD, FACOG, MCLD

SELF-ASSESSMENT QUESTIONS

7. List the various sources of gonadotropins used to produce the gonadotropin products that are available today.

2. Describe the relative purity of the highly purified and recombinant gonadotropins.

- J. Check the infectious risk for commercially available gonadotropins.**

4. Describe several of the clinical studies comparing recombinant and urinary gonadotropins.

3. Discuss the impact of gonadotropin source on the cost effectiveness of ART treatment cycles.

NOTES



Delphine Lévy, MD

PROFILE

A native of France, Dr. Lévy is currently assistant professor at Hôtel Dieu de Paris. She obtained a clinical fellowship in Medical Gynecology and Endocrinology in Paris. Dr. Lévy is also currently enrolled in a PhD program in Molecular Biology at the Institut Biomedical des Cordeliers (Paris Unit 305, Dr Jean Chambaut) in Paris. Her clinical research interest is in ovarian reserve testing and polycystic ovary syndrome (PCOS) endocrinology. Her basic research explores ovarian bioactivity and its role in the PCOS phenotype, the subject of her PhD thesis.

Dr. Lévy studied previously at Paris Descartes University, Paris, receiving a master's degree in Biomedical Ethics. She served a five-year residency in Endocrinology in Paris, and she received additional board certifications in Andrology (1993), and in Reproductive Medicine (2000). Between 1996 and 1999, she was a research fellow in Reproductive Endocrinology at the Center for Reproductive Medicine and Infertility at Weill Cornell Medical College of Cornell University (Dr. Rosenwaks), as well as with the Population Council (Dr. Indrani Bajjalal), at Rockefeller University in New York City.

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Delphine Lévy, MD

ABSTRACT

THE ROLE OF LH IN FOLLICULAR GROWTH AND OVULATION

Delphine P. Lévy,
Service de Gynécologie, Hôtel Dieu, Paris, France.
José M. Navarro
Instituto Vulliamina de Infertilidad, IVI Soria, Soria, Spain
Glenis L. Schwartzman
The Center for Reproductive Medicine and Infertility,
Weill Medical College of Cornell University, New York, USA.
Zey Rosenwaks
The Center for Reproductive Medicine and Infertility,
Weill Medical College of Cornell University, New York, USA.

Relatively little is known about the physiological roles of luteinizing hormone bioactivity (LH) during the follicular phase in humans. Until recently, follicular stimulation protocols had excluded both follicle stimulating and luteinizing hormones in an attempt to mimic the physiology of normal human folliculogenesis. However, many recent gonadotropin administration regimens have completely substituted LH bioactivity. The importance and the amount of LH necessary for optimal follicular stimulation are in fact still a matter of intense debate. Several animal and in vitro studies recently added to our understanding of the actions of androgens, estrogens, gonadotropins, and human on the follicle-oocyte unit, allowing a less speculative approach. Indeed, if androgens do not promote follicular atresia, if the LH directly influences oocyte quality or embryo growth, and if excessive LH levels are detrimental only on granulosa cells from polycystic ovaries, then the addition of LH to ovarian stimulation protocols might have beneficial effects. The careful study of the gonadotropin-releasing hormone analogs + recombinant gonadotropin stimulation regimens in vivo effects should soon permit a re-evaluation of the historical two-cell, two-gonadotropin hypothesis on which was based most of the protocol designs in the past years. These pharmacological tools may provide essential insights into the physiological roles of follicle stimulating and luteinizing

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hormones in human follicular development, and oocyte maturation, and give clinicians the valuable opportunity to not only individualize the ovarian stimulation protocols according to the patient's medical history in an effort to maximize oocyte yield, but also to improve oocyte and embryo quality, in order to evaluate further the impact of LH in ovarian stimulation, studied end-points should inevitably include fertilization rates, embryo growth rates and quality, implantation rates, embryo cryosurvival, and possibly blastocyst formation.

Our review of the recent literature has allowed us to formulate several reflections in view of the future directions of research. (4) There is no evidence-based clinical argument that the LH content of the available preparations for COV negatively affects the outcome of IVF treatments. (5) The importance of estrogen action on human granulosa cell function and oocyte maturation had been questioned in the discovery that the E2- β isozyme was highly expressed in the human ovary, and should now be re-evaluated and taken into account in study designs. (6) It is possible that a substantial number of normogonadotropic women are pharmacologically down-regulated by standard GnRH agonist stimulation, and could benefit from the addition of LH to their stimulation protocol. (6) New ovarian stimulation strategies are needed, including purified or recombinant FSH (with an optional bioform profile) to hCG, recombinant LH or hCG in varying amounts, tailored for the individual patient. (6) LH could improve follicle recruitment when low doses are administered during early follicular phase (hMG or hCG), and improve the control of the follicle cohort size and homogeneity when high dose administration is the late follicular phase during ovulation induction. (7) Future clinical trials comparing different stimulation protocols should be prospective, randomized and large enough to draw statistically valid conclusions.

REFERENCES

- [illegible]

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Service of Otorhinolaryngology
Maimonides

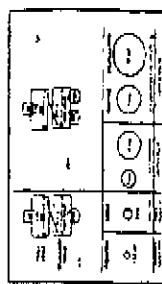
THE ACTION ON FOLLICLE AND OOCYTE

- * 1. *Neure "L'Esperance"*
- * 2. *Primrose Dale*
- * 3. *Le Petit Diable*
- * 4. *Clashed Tides*

LH ACTION ON FOLLICLE
AND OOCYTE

- 1. Direct Actions
- 2. Propaganda Actions
 - Leaflets
 - Demonstrations (Solidarity)

HUMAN POLYCLONAL ANTIBODIES



1. Nature Experiments

ITS NOT A MATTER OF IF YOU CAN AFFORD IT

- ESH about 170mmHg
 - Above 2nd stage Hypertension
 - Independent of LV wall thickness + EF
 - Normal LV wall thickness
 - • Number of Myocytes Increased
 - • Myocyte Fractionation Mass + Cytoplasm
- No correlation with EF alone

LHR INACTIVATING MUTATIONS

- Ovarian Atrophy
- All stages of Follicular Development
- Up to 10% of Normal FSH
- No Corpus Luteum
- Low LH Levels

Source: and the Journal of the Endocrine Society

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17 α -HYDROXYLASE DEFICIENCY

- Normal Follicular Development and Ovarian Size
- In the Premenopausal Period, T Levels
- 1/2 of Normal Ovarian and Ovarian Follicles
- 1/2 of Normal LH Levels
- 1/2 of Normal LH Levels
- 1/2 of Normal LH Levels
- 1/2 of Normal LH Levels
- 1/2 of Normal LH Levels

Source: and the Journal of the Endocrine Society

8

LH-RECEPTOR MIMICRY KNOCK-OUT

- Normal Follicular Development and Ovarian Size
- LH Levels at Birth
- LH Levels at Birth
- LH Levels at Birth
- LH Levels at Birth
- LH Levels at Birth
- LH Levels at Birth
- LH Levels at Birth

Source: and the Journal of the Endocrine Society

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LH-RECEPTOR KNOCK-OUT

- Female Mice, Puberty
- LH Levels at Birth
- LH Levels at Birth
- LH Levels at Birth
- LH Levels at Birth
- LH Levels at Birth
- LH Levels at Birth
- LH Levels at Birth

10

>>> 2. Primate Studies

11

3 β -HSD OR AROMATASE INHIBITION

- Approximately Normal Follicular Development
- 1/2 of Normal Ovarian
- 1/2 of Normal Ovarian
- 1/2 of Normal Ovarian
- 1/2 of Normal Ovarian
- 1/2 of Normal Ovarian
- 1/2 of Normal Ovarian
- 1/2 of Normal Ovarian

Source: and the Journal of the Endocrine Society

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OVARIAN STIMULATION

- Gonadotropins + GnRH + GnRH
- Gonadotropins + GnRH + GnRH
- Gonadotropins + GnRH + GnRH
- Gonadotropins + GnRH + GnRH
- Gonadotropins + GnRH + GnRH
- Gonadotropins + GnRH + GnRH
- Gonadotropins + GnRH + GnRH
- Gonadotropins + GnRH + GnRH

Source: and the Journal of the Endocrine Society

13

ROLE OF E2?

- Approximately Normal Follicular Development
- "Developmental" and Androgen Production
- Androgen Production Facilitates LH Surge
- 1/2 of Normal LH Levels
- 1/2 of Normal LH Levels
- 1/2 of Normal LH Levels
- 1/2 of Normal LH Levels
- 1/2 of Normal LH Levels

14

ANDROGEN AND FSH SYNERGY

- AR and FSH & GnRH
- AR and FSH & GnRH
- AR and FSH & GnRH
- AR and FSH & GnRH
- AR and FSH & GnRH
- AR and FSH & GnRH
- AR and FSH & GnRH
- AR and FSH & GnRH

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ANDROGEN RECEPTOR

- AR mRNA in GC
- AR mRNA in GC
- AR mRNA in GC
- AR mRNA in GC
- AR mRNA in GC
- AR mRNA in GC
- AR mRNA in GC
- AR mRNA in GC

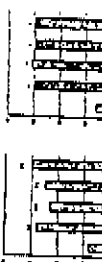
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LgP1 and LgP2 mRNAs GRANULOSA CELLS



17

LgP1 and LgP2 mRNAs GRANULOSA CELLS



18



Modern Day Gonadotropins In IVF

From Technology to New Clinical Data



Modern Day Gonadotropins In IVF

From Technology to New Clinical Data

**ANDROGEN ACTIONS
PRIMATE OVARY**

- Fertilization and Growth
- Testosterone
- Synergistic with FSH
- LH/FSH Ratio
- Androgen Pre-Treatment ?

19

PCOS GRANULOSA CELLS

ULTRASHORT ACTING

- ALR 23 to 10 day Follicular Diameter
- For Normal Women or Ovarian PCOs
- Adjuvant to LH/FSH for the Abnormality
- PCOs Patients

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>> 4. Clinical Trials

23

>> 3. In Vitro Data

20

PCOS GRANULOSA CELLS

INSULIN MODULATION OF LH ACTION

22

HYPOTHALAMIC-PITUITARY-ADRENAL

ADRENAL ACTIVITY

- (LH) increases +10% each 235 IU
- No Correlation in the 0 and 20 IU
- LH/FSH Ratio
- LH/FSH Ratio
- LH/FSH Ratio
- LH/FSH Ratio

24

PROLONGED GONADOTROPIN SUPPRESSION

PITUITARY SUPPRESSION

- Grouped by mid-follicular (LH)
- LH/FSH ratio > 4.0 (LH)
- Fertilization Rate, No of Embryos
- Mid-follicular LH/FSH Ratio
- LH/FSH Ratio
- LH/FSH Ratio

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PITUITARY SUPPRESSION

GONADOTROPIN SUPPRESSION

27

WHICH LH ?

29

PROLONGED GONADOTROPIN SUPPRESSION

PITUITARY SUPPRESSION

- Clinical Experience: The LH
- LH/FSH Ratio
- LH/FSH Ratio
- LH/FSH Ratio
- LH/FSH Ratio

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PITUITARY SUPPRESSION

GONADOTROPIN SUPPRESSION

28

CONTROL OF THE COHORT

30

SISATVHF-FIELD ONLY

[illegible]

WHAT IS THE "OPTIMAL" STIMULATION PROTOCOL?



CONCLUSION

- to Lyle, it means we are not the police-state ruled U.S. or the Islamic police state.

THE "PERFECT" STUDY??

- Large, homogeneous, Prospective, Randomized...
- 1 Single Primary Suppression Pit
- More End-Pain
- Side-effects Degrade

WHAT IS THE OBJECTIVE?



CONCLUSION

- **Laurel and the Human Ovary:**
It's only the beginning...
- **PCOS:** an insidious, relentless problem?
- **How can we monitor the quest?**
In Part...
In Part...

SELF-ASSESSMENT QUESTIONS

7. What is usually described as the two-cell, two-gonadotropin theory?
 - a. The pituitary cell population secreting both FSH and LH.
 - b. The physiological coupling of the GnRH hypothalamic secretion and the pituitary FSH/LH secretion.
 - c. The appearance of the LH receptors in the granulosa cells at mid-follicular phase.
 - d. The absence of aromatase activity in the theca cells.
 - e. The coupling of the theca and granulosa cells allowing the physiological production of estradiol by the ovary.
8. What are the predominant localization(s) of the estrogen receptor ER in adult expression in the human?
 - a. Endometrial glands.
 - b. Breast.
 - c. Granulosa cells.
 - d. Theca cells.
 - e. Brain.

- What are the androgen actions in the premenopausal ovary?
- Arousal of the premenopausal follicles
- Proliferation of the granulosa cells
- Synergy with FSH action on the primary and antral follicles
- Growth factor
- Testosterone production

1. d and e
2. c and e
3. b and c
4. a and e
5. a, b, and c

- d. What can be obtained when a hypogonadotropic hypogonadal woman is stimulated with recombinant human FSH alone?
 - a. Apparently normal karyogenesis
 - b. Oviduction
 - c. Normal estradiol production
 - d. Pregnancy
 - e. Oocyte maturation after during IVF procedure
- e. What experiments of nature are spontaneously hypogonadotropic?
 - a. 17 hydroxylase deficiency
 - b. Aromatase deficiency
 - c. 17,20 desmolase deficiency
 - d. 21 hydroxylase deficiency
 - e. LH-R mutation

NOTES

Michael P. Steinkampf, MD

PROFILE

Dr. Steinkampf is currently professor and director of the Division of Reproductive Biology and Endocrinology at the University of Alabama at Birmingham (UAB), and Director of the UAB Canine Biology Laboratory. A native of Louisiana, he received his undergraduate training at Louisiana State University. After obtaining a master's degree in chemistry at Princeton University, he attended LSU Medical School in New Orleans. Dr. Steinkampf completed his residency in OB/GYN at Parkland Hospital in Dallas, Texas in 1983, and he spent two additional years in at UT-Southwestern Medical School completing a fellowship in Reproductive Endocrinology, where he characterized the regulation of annotated P-450 gene expression in human granulosa cells.

Dr. Steinkampf has been on the faculty at UAB since 1987. His current research interests include management of ovulatory disorder and the optimization of culture conditions for human IVF. The author of over 100 publications, Dr. Steinkampf has received 20 research grants and is currently funded by the National Institutes of Health as a principal investigator in the National Collaborative Reproductive Medicine Network. He is co-holder of a patent describing a system of homologous tubal cells for embryo coculture.

Michael P. Steinkampf, MD

ABSTRACT

RESULTS OF FSH IVF CLINICAL TRIALS HIGHLY PURIFIED HUMAN-DERIVED FSH (BRAVELLE™) VS RECOMBINANT FSH (FOLLISTIM™) FOR IN VITRO FERTILIZATION

Background: Bravelle is a newly developed highly purified urofollitropin, containing 25 IU of FSH, with only 1-2% LH activity. Recently, a multicenter study was conducted to compare the efficacy and safety of Bravelle administered either subcutaneously (SC) or intramuscularly (IM); with Follistim SC for ovarian stimulation in women undergoing in vitro fertilization (IVF).

Materials and Methods: Eleven IVF programs participated in this randomized, controlled, open-label trial, enrolling women 18-39 years of age with infertility due to tubal damage, endometriosis (AFS I or II), or unexplained causes. All patients received heparin 9.5 mg/day SC for pituitary down regulation, and were then randomized to Bravelle SC, Bravelle IM, or Follistim SC at an initial dose of 225 IU/day for five days. Subsequent doses were individualized according to patient response, with a maximum dose of 450 IU/day, and no more than 12 days of stimulation. HCG (10,000 IU IM) was given when there were at least 3 follicles >16 mm diameter. Progesterone vaginal gel (Crinone, 8%) was begun after embryo transfer for luteal supplementation. The study was powered to detect a 20% between-group difference in the number of oocytes/cycle, the primary measure of efficacy. Secondary endpoints included serum estradiol levels, cycles with oocyte retrieval and embryo transfer, pregnancy rates, and adverse events.

Results: A total of 191 women were enrolled for this study. Of these, 129 were randomized to the three treatment arms. There were no significant differences among groups with respect to patient age, weight, or height, but there was a trend for greater body mass index in the Bravelle IM group (P=0.076). Cycle cancellation rates were comparable among the treatment groups, with 16% of women ultimately needing the criteria for hCG administration. There were no significant differences in mean peak serum estradiol levels among the three groups, although patients receiving Bravelle IM acquired slightly longer stimulation for adequate follicle development compared to Bravelle SC or Follistim SC. The mean number of oocytes retrieved were not significantly different among the groups (Bravelle SC: 14.1, Bravelle IM: 13.1, Follistim SC: 13.6; P=0.202), and the continuing pregnancy rates were also comparable (Bravelle SC: 44.6%, Bravelle IM: 44.5%, Follistim SC: 30.4%; P=0.222). The incidence of serious adverse events was not different among treatment groups, but patients who received Follistim SC reported more pain with injection than either group receiving Bravelle (P=0.03).

Conclusion: Bravelle SC and IM were at least as effective as Follistim in primary and secondary efficacy parameters, with similar safety profiles, but Bravelle produced less injection site pain compared to Follistim.



MOBILE, ALA. (AP) — A woman who was
in the car with a man who was shot
by police in the back of the head
last week is now in the hospital.
The woman, 34, was shot in the
back of the head by police in the
back of the head last week.

Michael P. Steinkamp, MD,
Professor of Medicine, Department of Obstetrics
Division of Reproductive Endocrinology & Infertility
University of Alabama at Birmingham
Birmingham, Alabama

BRAYELLE IN IVF-ET

- * A COMPARISON OF THE EFFICACY AND SAFETY OF GRAVELLE SC, BAYVILLER M4, AND POLIUNO SC IN IVF-ST

PRODUCT DESCRIPTION:
BRAVELLE

- LACK OF TOLERANCE FOR INFECTION, PARASITES
- TEND TO BE VERY PLUMPED ON THE HEAD OF FOLLOWS
- UP WITH LE ACTIVITY
- NOT NOT OF ECTABLE WITH DURING SEASON
- SUBJECT AREOUS OR INTRAMUSCULAR

**NDA PIVOTAL IVF STUDY:
99-04 BRAVELLE IN IVF-ET**

- PROSPECTIVE, RANDOMIZED, COMPARATIVE, PARALLEL-GROUP, MULTICENTER, OPEN-LABEL TRIAL.

POWER ANALYSIS TO
DETERMINE SAMPLE SIZE:
BRAVELLE IN IVF-ET

- 80% POWER WITH ALPHA = 0.05 (TAILED TEST) TO DETECT A 10% BETWEEN-GROUP DIFFERENCE
- REFERENCE TREATMENT: MEAN IN OCCYTES = 1400
- SAMPLE SIZE 40 PATIENTS PER TREATMENT

PRINCIPAL INVESTIGATORS AND
STUDY SITES: BRAYELLE MIVF.IT

Year	1950	1951	1952	1953	1954	1955	1956	1957	1958	1959	1960	1961	1962	1963	1964	1965	1966	1967	1968	1969	1970	1971	1972	1973	1974	1975	1976	1977	1978	1979	1980	1981	1982	1983	1984	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024	2025	2026	2027	2028	2029	2030	2031	2032	2033	2034	2035	2036	2037	2038	2039	2040	2041	2042	2043	2044	2045	2046	2047	2048	2049	2050	2051	2052	2053	2054	2055	2056	2057	2058	2059	2060	2061	2062	2063	2064	2065	2066	2067	2068	2069	2070	2071	2072	2073	2074	2075	2076	2077	2078	2079	2080	2081	2082	2083	2084	2085	2086	2087	2088	2089	2090	2091	2092	2093	2094	2095	2096	2097	2098	2099	2100																			
Population	1,000,000	1,050,000	1,100,000	1,150,000	1,200,000	1,250,000	1,300,000	1,350,000	1,400,000	1,450,000	1,500,000	1,550,000	1,600,000	1,650,000	1,700,000	1,750,000	1,800,000	1,850,000	1,900,000	1,950,000	2,000,000	2,050,000	2,100,000	2,150,000	2,200,000	2,250,000	2,300,000	2,350,000	2,400,000	2,450,000	2,500,000	2,550,000	2,600,000	2,650,000	2,700,000	2,750,000	2,800,000	2,850,000	2,900,000	2,950,000	3,000,000	3,050,000	3,100,000	3,150,000	3,200,000	3,250,000	3,300,000	3,350,000	3,400,000	3,450,000	3,500,000	3,550,000	3,600,000	3,650,000	3,700,000	3,750,000	3,800,000	3,850,000	3,900,000	3,950,000	4,000,000	4,050,000	4,100,000	4,150,000	4,200,000	4,250,000	4,300,000	4,350,000	4,400,000	4,450,000	4,500,000	4,550,000	4,600,000	4,650,000	4,700,000	4,750,000	4,800,000	4,850,000	4,900,000	4,950,000	5,000,000	5,050,000	5,100,000	5,150,000	5,200,000	5,250,000	5,300,000	5,350,000	5,400,000	5,450,000	5,500,000	5,550,000	5,600,000	5,650,000	5,700,000	5,750,000	5,800,000	5,850,000	5,900,000	5,950,000	6,000,000	6,050,000	6,100,000	6,150,000	6,200,000	6,250,000	6,300,000	6,350,000	6,400,000	6,450,000	6,500,000	6,550,000	6,600,000	6,650,000	6,700,000	6,750,000	6,800,000	6,850,000	6,900,000	6,950,000	7,000,000	7,050,000	7,100,000	7,150,000	7,200,000	7,250,000	7,300,000	7,350,000	7,400,000	7,450,000	7,500,000	7,550,000	7,600,000	7,650,000	7,700,000	7,750,000	7,800,000	7,850,000	7,900,000	7,950,000	8,000,000	8,050,000	8,100,000	8,150,000	8,200,000	8,250,000	8,300,000	8,350,000	8,400,000	8,450,000	8,500,000	8,550,000	8,600,000	8,650,000	8,700,000	8,750,000	8,800,000	8,850,000	8,900,000	8,950,000	9,000,000	9,050,000	9,100,000	9,150,000	9,200,000	9,250,000	9,300,000	9,350,000	9,400,000	9,450,000

INCLUSION CRITERIA: BRAVELLE IN IVF-ET

- * FUTURE FACTOR, CREDIT RISK IS IN PLACE FOR UNPLANNED INTEREST AT AT LEAST ONE YEAR
ACQUAIPR OPERATIONS CYCLES OF 30-35 DAYS
NORMAL HUMANITY PROTECTS INCLUDING DAY 100-150, 250 AND 170

TREATMENTS AND DOSING:
BRAVELLE IN IVF-ET

- DOWN REGULATE LEUKOCYTES
- MODIFY
- PITUITARY HORMONES
- INDIVIDUAL RESPONSE TO TREATMENT
- MAX GALT TREATMENT 400 IU
- MAX DURATION OF TREATMENT 12 DAYS
- 1 FOLLOW UP IN 10 MIN
- 1 MORE FOLLOW UP

MEASURES OF EFFICACY:
BRAVELLE IN IVF-ET

- THE**
• MEMBER OF SOCIETY
WILLIAM

**FLOW DIAGRAM OF TREATMENTS:
BRAVETTE IN DET**

MEASURES OF SAFETY;
BRAVELLE IN IVF ET

- ADVERSE EFFECTS**
HEART RATE INCREASE
 INCREASED HEART RATE AND VITAL SIGN
 MONITORING

Modern Day Gonadotropins in IVF

from Technology to New Clinical Data

PATIENT SUMMARY:
BRAVELLE IN IVF-ET

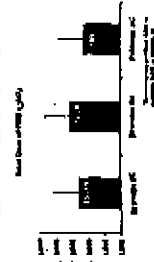
- 12 PATIENTS INBRED
- 116 NOT RANDOMIZED
- 118 FAILED TO DOWNREGULATE
- 119 BECAME PREGNANT BEFORE IN
- 120 ABNORMAL PAP TEST
- 121 HORMONAL FLUCT
- 122 RANDOMIZED INTENT-TO-TREAT POPULATION

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PATIENTS RECEIVING MCG:
BRAVELLE IN IVF-ET

	Group	Age	Weight	Height	Weight	Height
Control	100	35	160	5'6"	160	5'6"
Study	100	35	160	5'6"	160	5'6"
Total	200	70	320	11'2"	320	11'2"

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DOSE:
BRAVELLE IN IVF-ET

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DEMOGRAPHIC CHARACTERISTICS:
BRAVELLE IN IVF-ET

	Group	Age	Weight	Height	Weight	Height
Control	100	35	160	5'6"	160	5'6"
Study	100	35	160	5'6"	160	5'6"
Total	200	70	320	11'2"	320	11'2"

14

EL LEVELS:
BRAVELLE IN IVF-ET

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THERAPY DAYS:
BRAVELLE IN IVF-ET

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Modern Day Gonadotropins in IVF

from Technology to New Clinical Data

PRIMARY EFFICACY RESULTS:
BRAVELLE IN IVF-ET

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SECONDARY EFFICACY RESULTS:
BRAVELLE IN IVF-ET

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SAFETY: ADVERSE EVENTS:
BRAVELLE IN IVF-ET

	Group	Age	Weight	Height	Weight	Height
Control	100	35	160	5'6"	160	5'6"
Study	100	35	160	5'6"	160	5'6"
Total	200	70	320	11'2"	320	11'2"

23

SECONDARY EFFICACY RESULTS:
BRAVELLE IN IVF-ET

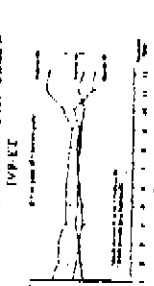
	Group	Age	Weight	Height	Weight	Height
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20

SECONDARY EFFICACY RESULTS:
BRAVELLE IN IVF-ET

	Group	Age	Weight	Height	Weight	Height
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Study	100	35	160	5'6"	160	5'6"
Total	200	70	320	11'2"	320	11'2"

22

INJECTION SITE PAIN:
QUESTIONS: DATA: BRAVELLE IN IVF-ET

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SUMMARY: BRAYELLE IN IVF-ET

- BRAYELLE SC AND DA WERE AT LEAST AS EFFECTIVE AS FOLLISTIM IN PRIMARY AND SECONDARY EFFICACY PARAMETERS
- BRAYELLE SC AND DA FREQUENTLY BATTER FOR ONE CYCLE MORE EFFECTIVE COMPARED TO FOLLISTIM SC

25

CONCLUSION

- FEMOR'S BRAYELLE, ADMINISTERED SC OR DA IS A HIGHLY EFFECTIVE AND WELL TOLERATED POLLUTANT THAT COMPARED TO FOLLISTIM PRODUCES LESS INJECTION-SITE PAIN

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SUMMARY: BRAYELLE IN IVF-ET (CONT'D)

- SAFETY PROFILES WERE SIMILAR ACROSS TREATMENT GROUPS
- BRAYELLE SC AND DA PRODUCED SIGNIFICANTLY LESS INJECTION-SITE PAIN COMPARED TO FOLLISTIM SC

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Michael P. Steinkampf, MD

SELF-ASSESSMENT QUESTIONS

1. Brayelle is a newly developed form of:

- Recombinant FSH
- Urofollitropin
- Human chorionic gonadotropin
- Progesterone
- Gonadotropin-releasing hormone

2. The amount of LH in Brayelle is:

- 0.1-0.2%
- 1-2%
- 10-20%
- 30%

3. The primary efficacy parameter in the recent multicenter study comparing Brayelle SC, Brayelle IM, and Follistim SC was:

- Pregnancy rates
- Number of oocytes retrieved
- Number of mature oocytes retrieved
- Number of follicles produced
- Injection site pain

4. In this study, the number of oocytes obtained with Brayelle SC was:

- Comparable to Brayelle IM and Follistim SC
- Significantly less than Brayelle IM and Follistim SC
- Significantly greater than Brayelle IM and Follistim SC
- Not calculated

5. In this multicenter study, injection site pain with Follistim was:

- Less than with Brayelle SC or IM
- Greater than with Brayelle SC or IM
- Not significantly different from Follistim per protocol
- Not reported by patients

Steinkampf
Answers

1. b
2. b
3. b
4. a
5. b

Modern Day Gonadotropins In IVF

NOTES

From Technology to New Clinical Data

Modern Day Conadotropics In IVF

NOTES

FP1027503

Richard P. Marra, MD

PROFILE

Dr. Richard P. Marra is a board certified Reproductive Endocrinologist. He studied medicine and trained in Obstetrics and Gynecology in Texas before moving to Southern California to study Reproductive Endocrinology. While at the University of Southern California (USC), he developed one of the country's first IVF programs. He is internationally recognized for his contributions to the development of IVF. Dr. Marra is currently the director of the Center for Assisted Reproductive at Santa Monica UCLA Medical Center. He is on the board of numerous medical and scientific organizations and is a prominent figure in the national and international infertility community. He has recently published a book for couples called "Dr. Richard Marra's Fertility Book".

Richard P. Marra, MD

ABSTRACT

STABILITY DATA AND MIXED PROTOCOL RESULTS

CLINICAL OUTCOME WITH DIFFERENT RATIOS OF FSH AND LH FOR CONTROLLED OVARIAN HYPERSTIMULATION FOR IN VITRO FERTILIZATION

From the early days of in vitro fertilization and embryo transfer (IVF-ET) hundreds of clinical studies have been performed with combinations of recombinant and urinary gonadotropin and GnRH agonists and antagonists for controlled ovarian hyperstimulation (COH). Even though standardized protocols are utilized in some centers, in most other centers individualization of ovarian stimulation is attempted by utilizing the clinical history of the patient (i.e. age, E2/FSH ratios, prior stimulation outcome, etc.). For example, in our center, results of individualized stimulation protocols are based upon:

- a) Prior stimulation outcome;
- b) Patient age;
- c) Day two FSH/E2 levels, and visualization of ovarian status by day two ultrasound.

In patients 40 years of age and older, pregnancy rates have ranged between 12 and 38% in almost 1,000 patients stimulated with GnRH agonists and urinary and/or recombinant FSH with or without hMG over a five-year period. The highest pregnancy rates/cycle have occurred with down regulation with GnRH agonist and urinary FSH/hMG for COH. A similar pattern was seen in approximately 800 patients over age 40 during the same 5-year time period.

Recently, a prospective randomized clinical trial performed by Ferring Pharmaceuticals has compared varying ratios of highly purified urinary FSH (Puregon) and hMG (Repronex) administered as a single SC injection following down regulation and COH for IVF-ET. The subjects were randomly assigned to three groups, A, B, and C. Subjects in Group A received FSH and LH in a ratio of 2:1.

Subjects in Group B initially received FSH and LH in a 3:0 ratio, which shifted to a 2:1 ratio after day 5 of stimulation. Group C received an initial FSH and LH ratio of 3:1, which could be increased to 4:1, 5:1 or 6:1 as needed. Preliminary results demonstrated that patients <34 years of age who were assigned to Group A had higher E2 levels and an increased number of oocytes than those subjects assigned to Groups B and C. The ongoing pregnancy rate and the multiple pregnancy rate was highest in Group A. Patients over age 34 did not demonstrate a similar trend, but the number of completed cycles are too few to perform meaningful statistical analysis at the present time.

In conclusion, FSH:LH ratios of 2:1 or 3:1 may give an advantage in oocyte number, oocyte quality and pregnancy outcome.

From Technology to New Clinical Data



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3 Anatomical Subjects Attending Hermann
 Fricke, GmbH
 Grossschloppe Holzing Hermann, Agents
 Leipzig, Germany
 Grossschloppe Holzing Hermann, Architects

- Prior ovulation cycles
- Oocyte number and quality in prior IVF
- Day 2 Estradiol/FSH levels
- Visualization of ovary volume and primary follicle number by ultrasound

[illegible]

Year	1994	1995	1996	1997
1994	10.0	10.0	10.0	10.0
1995	10.0	10.0	10.0	10.0
1996	10.0	10.0	10.0	10.0
1997	10.0	10.0	10.0	10.0

Study Design: Prospective, Randomized, 3-arm, Parallel Group

Subjects: 31

Age: 18-33 years

Inclusion Criteria: (OR, 4.8 years)

A. 1-1
B. 2-0 to 1-1
C. 2-1

- **Primary Efficacy Assessment:**
 - Number of cycles retrieved per cycle
- **Secondary Efficacy Assessment:**
 - Peak Serum E_2
 - Percentage of Clinical and Lingering Proliferations

- Unborn (current)
- Non-smoker age 34-40
- Regular ovulation (24-35 day cycle)
- Fertility of at least one year with normal male or use of donor sperm
- Normal uterus by HSG, IUS, or some HSG within 1 year

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- Informed consent
- Non-smoker age 18-35
- Regular ovulation (24-35 day cycle)
- Infertility of at least one year with normal male or base of disease sperm
- Menstrual uterus by HSG, HSC, or sono HbG within 3 years.

A Randomized, Comparative 3-Arm, Parallel Group, Open-Label, Multicenter Study of the Efficacy and Safety of Bravecto SC (Fluralaner FSD) and Reproject SC when Combined in the Same Syringe with Contraceptive or Sequential Dose Rates in Female Patients Undergoing In-Vitro Fertilization (IVF)

For more information, call 1-800-368-7233. In New York, call 212-633-1300. Fax 212-633-1301. E-mail: info@hugoboss.com

FP1027505

Modern Day Gonadotropins In IVF

Exclusion Criteria:
2000-04 & 05

- Clinically relevant if symptomatic disease
 including pregnancy
 Any interference with menstruation/ovulation
 Pregnancy or breast feeding 3 months prior
 to screening
 BMI ≥ 34
 More than 3 failed ART cycle
 Abnormal uterine bleeding
 Drug, tobacco, alcohol, substance
 Chronic stable therapy or recent treatment

2000-04 & 05

1. Day with Day 1-4
1. Longitudinal to 2.5% longitudinal
Responsible to treatment group A, B, C
Final stage Day 1-4
On Day 1 - longitudinal to 2.5% longitudinal
Group B - 1.0 day, longitudinal to 2.5% longitudinal
Group C - 1.0 day, longitudinal to 2.5% longitudinal

FREEMAN & DENIG:
7000 24th St. SE

- ^a Maximum FBS dose / day = 450 U/L.
- ^b Maximum duration of stimulation 15 days.
- ^c hCG 10,000 U/L / 1 mL when three (3) follicles ≥ 14 mm (3 phase measurement).
- ^d Oocytes retrieved 3-6 hours after hCG.

Insurance of Safety: 2004 & 05

- Infants**
PE₂ and US results
PPE and vehicle
Incident involving
SAD were events
Injections also peak : daily pattern
on these numbers

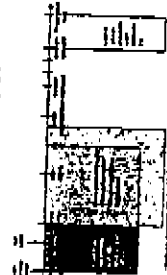
Principal Investigators and Study

Country Name & Location	Year(s) of Study
Belgium (Brussels)	1970-1971
Belgium (Brussels)	1972-1973
Belgium (Brussels)	1974-1975
Belgium (Brussels)	1976-1977
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Belgium (Brussels)	2214-221

Principal Investigators and Study

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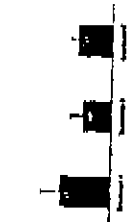
FLOW DIAGRAM OF TREATMENTS:
 RECOVERING FROM OCEAS



ATIENTS RECEIVING PCG:

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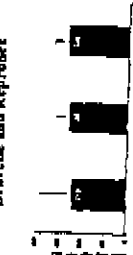
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DEMOGRAPHIC CHARACTERISTICS:
PROTOCOL 100-PM; AGE 23-33 YEARS

[illegible]

Problem Daily Dose FSH:
2000-05



MATH LEVEL: 2000-04



Modern Day Gonadotropins In IVF

From Technology to New Clinical Data

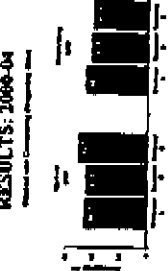


Modern Day Gonadotropins In IVF

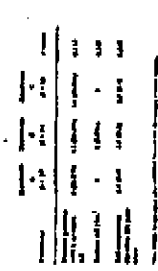
From Technology to New Clinical Data

PRIMARY EFFICACY RESULTS:
2000-04

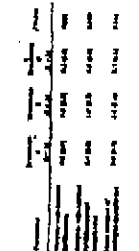
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SECONDARY EFFICACY
RESULTS: 2000-04

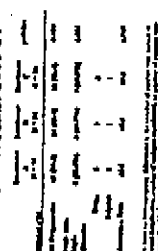
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SAFETY: ADVERSE EVENTS
2000-04

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SECONDARY EFFICACY
RESULTS: 2000-04

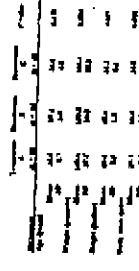
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SINGLETON/MULTIPLE
PREGNANCIES: 2000-04

28

Injection-site Pain Questionnaire:
2000-04

30

DEMOGRAPHIC
CHARACTERISTICS:
2000-05

31

PATIENTS RECEIVING RCG:
2000-05

32

Mean Total Dose LH: 2000-05



34

Mean Daily Dose FSH: 2000-05
Bravecto and Bravecto

33

E₂ LEVELS: 2000-05

35

PRIMARY EFFICACY RESULTS:
2000-05

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Modern Day Gonadotropins in IVF

From Technology to New Clinical Data

SECONDARY EFFICACY
RESULTS: 2000-05

	Group	Mean	SD	95% CI	P
Overall	Control	1.74	0.14	1.46-2.02	0.0001
	Study	2.14	0.14	1.86-2.42	
Subgroup	Control	1.74	0.14	1.46-2.02	0.0001
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Injection-site Pain Questionnaire:
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SAFETY AND TREATMENT
TOLERATION: 2000-05

	Group	Mean	SD	95% CI	P
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	Study	2.14	0.14	1.86-2.42	
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Days of Luteal Phase Down-Regulation to
Start of Stimulation: 2000-05

42

Modern Day Gonadotropins in IVF

From Technology to New Clinical Data

Mean Total Dose FSH: 2000-04/05
Brevets and Reproces

43

Mean Peak Serum E₂ Levels:
2000-04/05

45

Continuing Pregnancy Rates:
2000-04/05

47

Mean Days of Therapy:
2000-04/05

44

Mean Number Oocytes
Retrieved: 2000-04/05

46

Summary

- Individualized individualized protocols may increase success rates in IVF.
- Preliminary results suggest that rates of FSH, E₂ may be optimized at 2.14 or 2.14 units for treatment for gonadotropin and quality in all age groups.
- Rates of FSH, E₂ at 2.14 level may be lower than 2.14 in 2.14 or 2.14 age group.

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Conclusions

- Subcutaneous injection of recombinant hCG (Ovidrel) has been demonstrated to be safe and effective in the treatment of women with luteal phase defects.
- Even though the rates of pregnancy and live birth are similar between women treated with subcutaneous hCG and women treated with intramuscular hCG, the rates of side effects are lower in women treated with subcutaneous hCG.
- Patients in the 24-40 age group demonstrated a higher rate of pregnancy and live birth when treated with subcutaneous hCG compared to intramuscular hCG. This finding needs to be confirmed in a larger patient group.

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Richard P. Marrs, MD

SELF-ASSESSMENT QUESTIONS

1. Factor(s) that may improve selection of proper stimulation protocols for IVF include(s):
 - a. Patient age
 - b. Prior stimulation response
 - c. Day 2 E2 / FSH levels
 - d. Day 2 OHCA level
 - e. a, b, and c
 - f. all of the above
2. Closte between down regulating, Base-up or non-Lupron antagonist protocols is best determined by:
 - a. Patient cycle length
 - b. Day 2 E2 / FSH levels
 - c. Adrenal profile
 - d. Day 2 ultrasound of ovaries for determination of # follicles and/or ovarian volume
 - e. a and c
 - f. b and d
3. Use of highly purified FSH (Bravelle) with hMG (Reprovas) demonstrated:
 - a. Good patient compliance
 - b. Low incidence of adverse events
 - c. No significant differences in injection site pain with any FSH/LH ratio
 - d. a and c
 - e. All of the above
4. All three ratios of FSH:LH demonstrated similar stimulation and pregnancy outcomes?
 - a. True
 - b. False
5. In a small group of IVF cycles Bravelle/Reprovas demonstrated an excellent pregnancy rate in all three ratios?
 - a. True
 - b. False

Answers

1. e
2. f
3. e
4. a
5. a

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